A VOLTAMMETRIC STUDY OF SOME ACTIVE INGREDIENTS IN COUGH DRUGS

by

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ABSTRACT

A simple method for the determination of active components in cough drugs using differential pulse voltammetry has been developed. The proposed method involves the use of a home - made glassy carbon electrode as the working electrode and the Britton Robinson buffer as the supporting electrolyte. The active components analysed were chlorpheniramine maleate, guaifenesin and phenylephrine hydrochloride, and the signals were observed at +0.675 V, +1.055 V and +0.560 V versus the saturated calomel electrode (SCE), respectively. The optimum experimental conditions and the effect of interfering substances were also reported. The method was applied to determine the content of these compounds in several common cough drugs.

The application of the voltammetric method for the simultaneous determination of two or more active components was attempted and found not to be satisfactory.

The simultaneous determination of five common active ingredients in cough mixtures by high performance liquid chromatography was also studied and reported in the Appendix.

CONTENTS

Chapter I : INTRODUCTION

Voltammetry is a group of electro-analytical methods which is based on the unique characteristic of the current-voltage curves. The working electrodes in voltammetry are generally having small surface area and referred as micro-electrodes. Polarography, which is a widely used voltammetric method, differs from others in that the dropping mercury electrode (DME) is served as a micro-electrode.

In 1934, Ilkovic derived the following equation relating various parameters that determined the diffusion currents using a dropping mercury electrode [1].

$$i = 607\ nD^{1/2} m^{2/3} t^{1/6} C$$

where i : time-average diffusion current during the life-time of a drop (μA)

n : number of faradays per mole of reactant

D : diffusion coefficient

m : rate of mercury flow (mg/s)

t : drop time (s)

C : concentration of the reactant (mmole/l)

Direct current polarography is the classical and the simplest method in which the voltage scan is a linear ramp as shown in Figure I-1. The current flow at a dropping mercury electrode is measured as a function of voltage. A typical current-voltage curve is given in Figure I-2 [2].

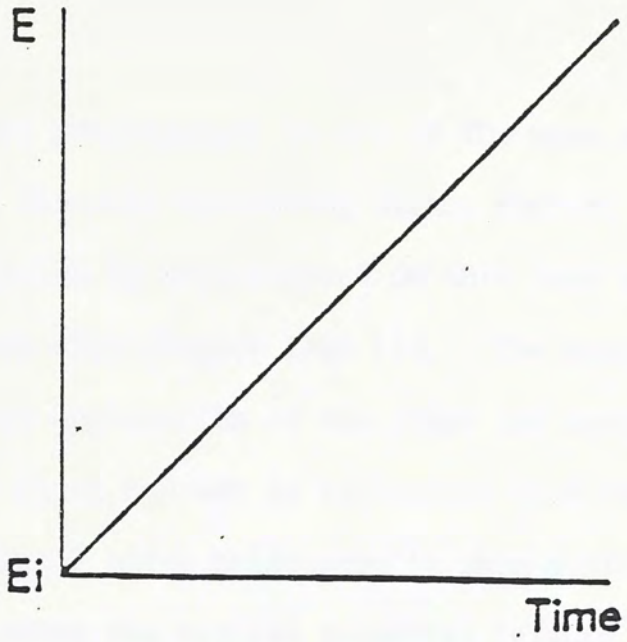


Figure I-1 : Programming waveform for direct current polarography

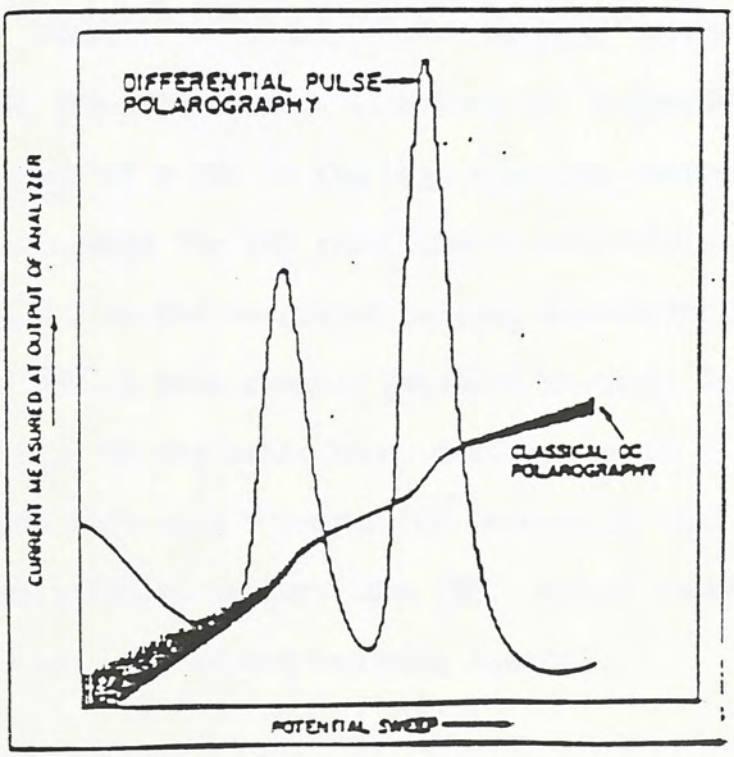


Figure I-2 : A comparison of polarograms between d. c. and d. p. polarography

Differential pulse polarography is one of the most sensitive technique in which a linearly increasing direct current ramp is applied and a fixed pulse is superimposed on this ramp near the end of the life of each drop (Figure I-3) [3]. The current flow is measured just before application of the pulse and again at the end of the pulse. The first current is subtracted from the second current. The differential pulse polarogram is thus a plot of the current difference against the applied potential (Figure I-2).

The curve is analogous to the derivative of the direct current polarogram, and the peak height is directly proportional to the concentration of the analyte.

Polarography became a widely used method with the application of the dropping mercury electrode by Heyrovsky in 1922 [4]. The advantage of a DME is the high hydrogen overvoltage so that the voltage range for the reduction is relatively wide and it is about -2.7 V vs the saturated calomel electrode (SCE). The reduction at a DME is thus roughly parallel to those reduced by metallic sodium [5]. On the other hand, mercury cannot be used at potentials larger than +0.4 V versus SCE because of the ease of its anodic dissolution as mercury ions [6], which makes it possible to oxidize only the strong reducing agents.

Most of the substitutes for the dropping mercury electrode are solid electrodes. In 1962, starting from phenolic resins, Yamada and Sato prepared gas-impermeable carbon, which was known

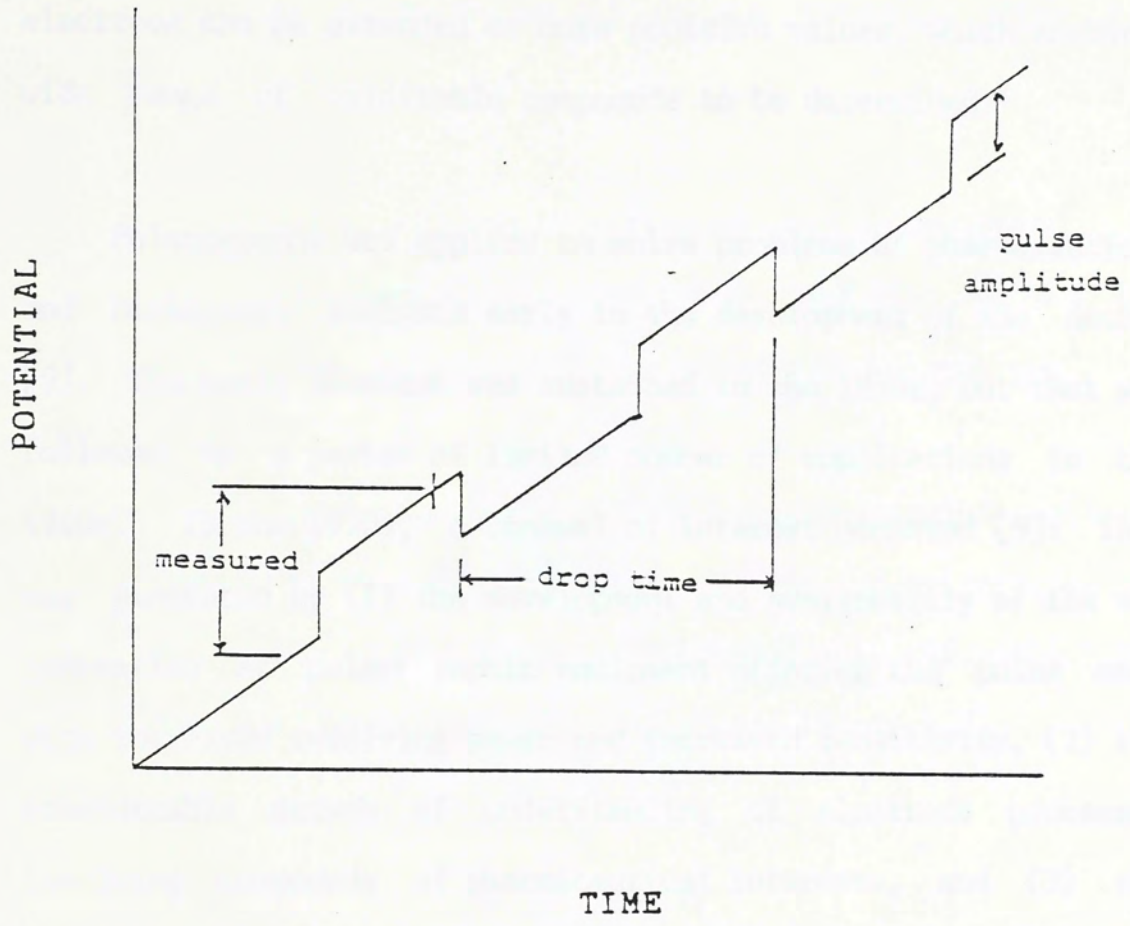


Figure I-3 : Programming waveform for differential pulse polarography

as glassy carbon [7]. The glassy carbon electrodes were applied for the first time in electroanalytical chemistry by Zittel and Miller [8], who showed that the usable potential range of the electrode can be extended to more positive values, which enable a wide range of oxidizable compounds to be determined.

Polarography was applied to solve problems of pharmaceutical and biological analysis early in the development of the method [9]. The early interest was sustained in the 1950s, but that was followed by a period of limited number of applications in the 1960s. In the 1970s, a renewal of interest occurred [9]: This was generated by (1) the development and availability of the new generation of polarographic equipment offering the pulse mode with increased resolving power and increased sensitivity, (2) the considerable growth of understanding of electrode processes involving compounds of pharmaceutical interests, and (3) the application of waves due to oxidation processes, thus making the technique applicable to a wider range of pharmaceutical preparations.

A remarkable advantage of polarographic methods in the analysis of formulations such as injections, tablets, dragees, oily solutions, ointments etc is that the excipients do not interfere to such large extent as they do in other methods. Dragees, e.g., can often be analysed in the presence of insoluble tablet materials.

Another example is the assay of fluocinolone acetonide in creams, gel and ointments by differential pulse polarography [10]. It was found that commonly used preservatives and bases in creams and gelling agents in gel did not interfere with the direct determination.

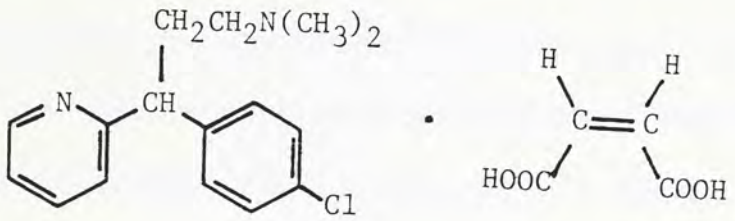
The acceptance of the significance of polarography and voltammetry in pharmacy can be seen by the fact that the USP XX [11] quotes 16 drugs and formulations assayed by polarography.

The assay procedure to determine chlorpheniramine maleate and guaifenesin in pharmacopoeia is based upon the extractions of analyte with ether or chloroform, followed by the measurement of the absorbance at wavelength 265 nm [12] and 276 nm [13] respectively.

The purpose of the present work was to determine chlorpheniramine maleate, guaifenesin and phenylephrine in cough drugs by differential pulse voltammetry. The details of the assay will be discussed in Chapters II to IV, respectively. The results were compared with those obtained using an established method. The simultaneous determination of these ingredients will be discussed in Chapter V. The chromatographic behaviour of five common ingredients of cough-cold liquids was examined by using the reverse-phase, ion-pair high-performance liquid chromatography, and the results were reported in the Appendix.

Chapter II : THE DETERMINATION OF CHLORPHENIRAMINE MALEATE

Chlorpheniramine maleate, a pyridine-derived antihistamine, is marketed in a variety of pharmaceutical preparations, and its structure is shown below :



The structure of chlorpheniramine maleate

A Pharmacopoeial method [12] [13] [14] is available for the assay of chlorpheniramine maleate in its pure form and in formulation tablets, syrups and injections. In this method, it is determined spectrophotometrically by measurement of its absorbance at 264-265 nm in aqueous solutions after extraction with dilute sulphuric acid. The drug has also been determined by other methods such as spectrophotometry [15] [16], gas chromatography [17] , and non-aqueous titration [18]. Most of these methods are not very sensitive, but time-consuming separations of the drug from complex mixtures are usually required before the determination.

The polarographic behaviour of chlorpheniramine maleate under both direct current and alternating current polarography was first investigated by Jacobsen and Hogberg [19]. Chlorpheniramine maleate in tablets was determined using d.c.

polarography with 0.2 M sulphuric acid as the supporting electrolyte.

The aim of the present work was to determine chlorpheniramine maleate using differential pulse voltammetry. Differential pulse was chosen because of its low detection limits and easily interpreted peak responses. The first part of the work was to study the differential pulse polarographic behaviour of chlorpheniramine maleate in 0.2 M sulphuric acid using a dropping mercury electrode (DME). The latter part was to investigate the differential pulse voltammetric behaviour of chlorpheniramine maleate in Britton-Robinson buffer using a glassy carbon electrode. The British Pharmacopoiea method was used as a parallel check for the results obtained voltammetrically.

(A) DIFFERENTIAL PULSE POLAROGRAPHIC METHOD

INSTRUMENTATION

A Princeton Applied Research Corporation (PAR) model 174A polarographic analyzer was used with a PAR model 303 SMDE electrode assembly with built-in Ag/AgCl reference electrode and platinum wire as counter electrode.

REAGENTS

All reagents used were of analytical grade, and 0.2 M sulphuric acid was used as the supporting electrolyte.

PROCEDURE

An aliquot (10 ml) of the supporting electrolyte (0.2 M sulphuric acid) was transferred by a pipette into a clean, dry cell, and a spike containing a small measured volume of chlorpheniramine maleate solution was then added. The peak height for the signal of chlorpheniramine maleate at -0.775 V vs the Ag/AgCl electrode for each solution was measured with the following instrument settings :

PAR 174A Polarographic Analyzer

Mode	Differential Pulse
Display direction	+ve
Initial potential	-0.4 V
Drop time	1 sec
Current range	varying
Scan rate	2 mV/sec
Scan direction	-ve
Modulation amplitude	50 mv
Low pass filter	OFF

PAR 303 Static Mercury Drop Electrode

Mode	DME
Drop size	medium

The tablets were grounded and about 0.2 g of the powder was accurately weighed and transferred to a 100 ml volumetric flask and 0.2 M sulphuric acid was added. The flask was shaken and its content was diluted to the mark with 0.2 M sulphuric acid. A suitable amount of the suspension was transferred to the polarographic cell. The amount of chlorpheniramine maleate present in the original sample was determined by the standard-addition calibration employing the linear least-square fitting technique.

RESULTS AND DISCUSSION

(1) Optimisation of parameters of the polarographic analyzer

In differential pulse polarographic determinations, the signal of the analyte depends on such parameters of the instruments as the modulation amplitude, drop time, and scan rate. The effect of drop time, modulation amplitude and scan rate on the peak height of chlorpheniramine maleate dissolved in 0.2 M sulphuric acid was investigated in order to determine the optimum values to be used for the determination of this compound. In each measurement, five readings were recorded and then averaged, and the relative standard deviation was calculated.

(i) Drop time

Table II-1 shows that the peak height increases with decreasing drop time. Although the signal with a drop time of 0.5 second was the highest, yet the reproducibility was not good. As a result, the drop time of 1 sec was chosen.

Table II-1 : Effect of drop time on the peak height of 17.5 ppm chlorpheniramine maleate measured with a DME at -0.775 V vs Ag/AgCl electrode, the modulation amplitude being 50 mV and scan rate 2 mV/s

Drop time (s)	Peak height (μA)	Average (μA)	Relative standard deviation
0.5	2.150 2.250 2.375 2.500 2.325	2.320	5.67 %
1.0	1.850 1.850 1.925 1.950 1.900	1.895	2.36 %
2.0	1.400 1.475 1.450 1.500 1.500	1.465	2.86 %

(ii) Modulation amplitude

The results from Table II-2 show that the larger the modulation amplitude, the higher would be the peak height. The modulation amplitude of 50 mV was chosen because of the smallest relative standard deviation and the large enough peak height obtainable.

Table II-2 : Effect of modulation amplitude on the peak height of 17.5 ppm chlorpheniramine maleate measured with a DME at -0.775 V vs Ag/AgCl electrode, the drop time being 1 sec and scan rate 2mV/s

Modulation amplitude (mV)	Peak height (μA)	Average (μA)	Relative standard deviation
10	0.250 0.300 0.350 0.325 0.300	0.305	12.16 %
25	0.850 0.875 0.850 0.850 0.800	0.845	3.24 %
50	1.850 1.875 1.900 1.850 1.900	1.875	1.33 %
100	3.875 3.650 3.625 3.950 3.850	3.790	3.81 %

(iii) Scan rate

The result from Table II-3 shows that the faster the scan rate, the smaller peak height and larger relative standard deviation would be obtained. However, the peak heights for scan rates of 1 mV/S and 2 mV/s were similar. The scan rate of 2 mV/s was chosen because shorter time was required for each run.

Table II-3 : Effect of scan rate on the peak height of 17.5 ppm chlorpheniramine maleate measured with a DME at -0.775 V vs Ag/AgCl electrode, drop time being 1 sec and modulation amplitude 50 mV

Scan rate (mV/s)	Peak height (μA)	Average (μA)	Relative standard deviation
1	1.850 1.850 1.875 1.850 1.900	1.865	1.20 %
2	1.850 1.850 1.825 1.875 1.900	1.860	1.53 %
5	1.750 1.750 1.700 1.725 1.675	1.720	1.90 %
10	1.300 1.325 1.275 1.250 1.350	1.300	3.04 %

In conclusion, the optimum parameters for operation were found to be modulation amplitude, 50 mV; drop time, 1 sec; and scan rate, 2 mV/sec. The peak potential would then be -0.775 V vs Ag/AgCl electrode, and a typical curve was shown in Figure II-1.

(2) Precision and Calibration Curve

The precision of the polarograhic method was assessed, where the relative standard deviation for five replicate determinations of a 10 ppm standard chlorpheniramine maleate solution was found to be 2.25 % (see Table II-4).

Table II-4 : Test of the precision for the determination of chlorpheniramine maleate (10 ppm) by differential pulse polarography using a DME

Trial number	Peak height (μA)
1	0.950
2	0.950
3	0.900
4	0.925
5	0.925
Average :	0.930 μA
Standard deviation :	0.02 μA
Relative standard deviation :	2.25 %

The reproducibility of the method is satisfactory.

A typical calibration graph is illustrated in Figure II-2, and the relavant data for this graph were shown in Table II-5.

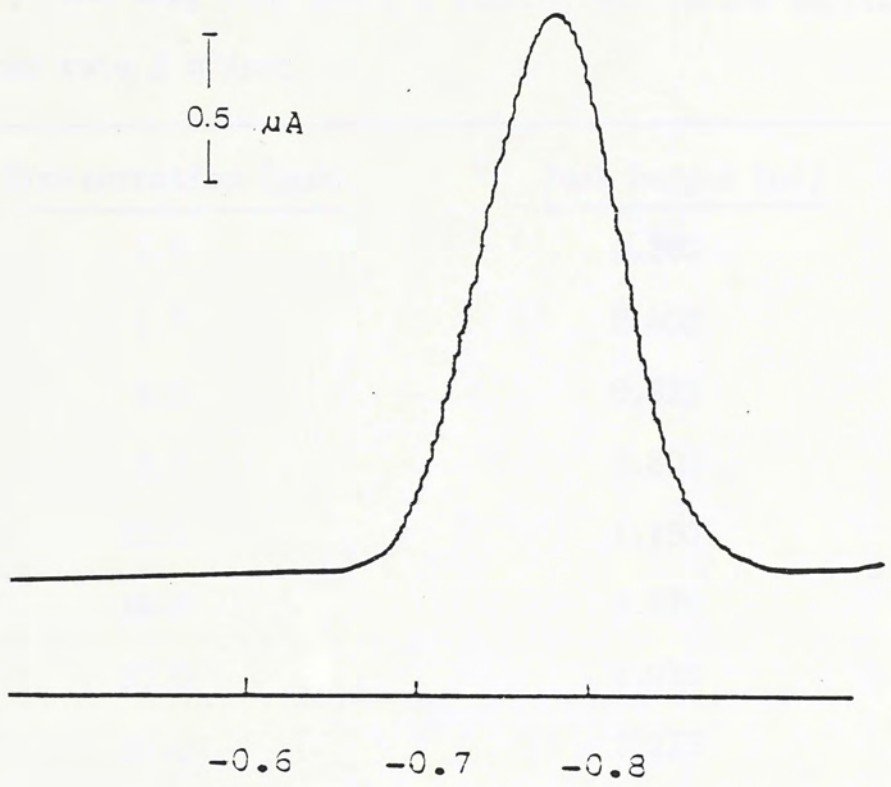


Figure II-1 : Signal of chlorpheniramine maleate (17.5 ppm) in 0.2 M sulphuric acid measured with a D M E vs Ag/AgCl electrode at a drop time of 1 s, scan rate of 2 mV/s and modulaion amplitude of 50 mV

Table II-5 : Data for the calibration grahp of chlorpheniramine maleate in 0.2 M sulphuric acid measured at -0.775 V vs Ag/AgCl electrode, the drop time being 1 second, modulation amplitude 50 mV and scan rate 2 mV/sec.

Concentration (ppm)	Peak height (μA)
2.0	0.200
4.0	0.400
6.0	0.625
8.0	0.800
12.0	1.150
16.0	1.575
20.0	1.950
24.0	2.275

The graph in Figure II-2 was linear for chlopheniramine maleate concentrations over the range of 2-24 ppm.

(3) Results for the Determination of Chlorpheniramine Maleate in Tablets

The content of chlorpheniramine maleate in tablets was determined by the differential pulse polarographic method using standard addition calibration. The results are summarised in Table II-6 and the results obtained by the established the B. P. method are also included for comparison. Each determination was done in duplicate.

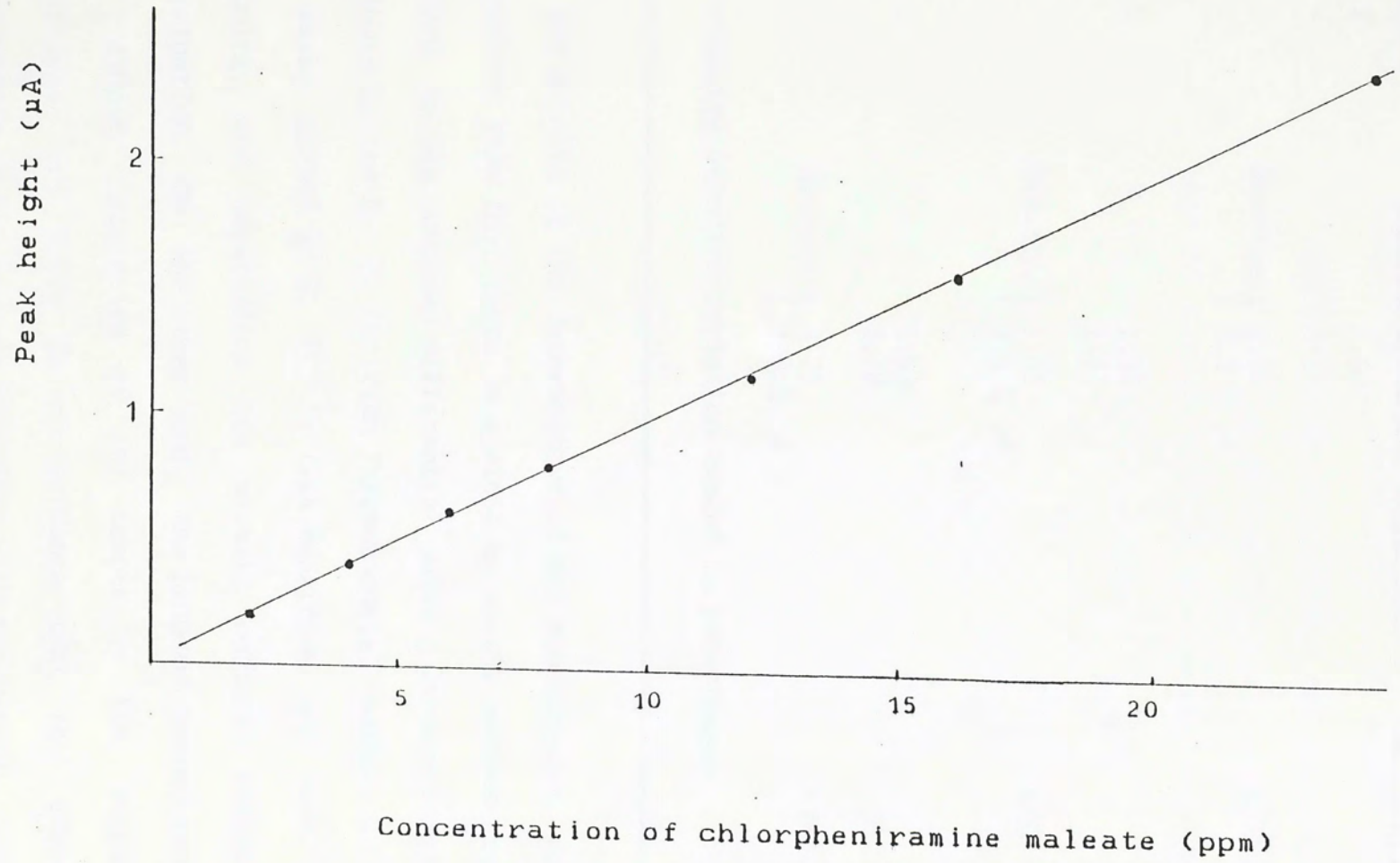


Figure II-2 : Calibration curve of chlorpheniramine maleate

Table II-6 : Determination of chlorpheniramine maleate in tablets.

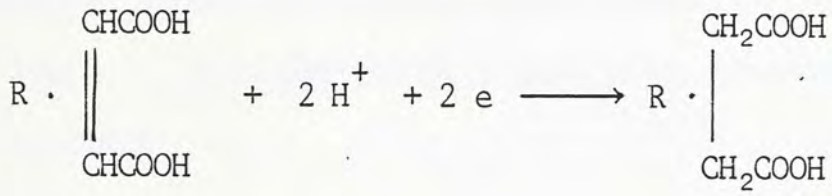
Sample	Chlorpheniramine maleate content (μg/tablet)	
	Proposed polarographic method	by B. P. method
1	1.98 1.89	
	Average : 1.94 (3.3 %)*	2.02
2	1.89 1.81	
	Average : 1.85 (3.1 %)*	1.92
3	3.63 3.78	
	Average : 3.72 (2.9 %)*	3.88

* relative standard deviation quoted in parentheses

The results of the determintion of chlorpheniramine maleate in tablets show that there is a close agreement between results obtained by the proposed differential pulse polarographic method and those by the B. P. (British Pharmacopoeia) method. In fact, the assay method in B. P. is less sensitive and more time-consuming, and separations are usually required before the determination. On the other hand, the proposed method requires only simple dissolution of the sample in the supporting electrolyte and there is no evidence that the insoluble constitutents in the tablets interfere with the method, and they need not be removed. Thus the proposed method is much simpler and more rapid than the B. P. method.

(B) DIFFERENTIAL PULSE VOLTAMMETRIC METHOD USING GLASSY CARBON ELECTRODE

In the polarographic determination of chlorpheniramine maleate using a dropping mercury electrode, the reduction wave had been reported to be similar to that of maleic acid [20]. And the reduction wave of chlorpheniramine maleate was thus proposed to be due to the following reaction [21].



The effect of maleic acid on the peak height of chlorpheniramine maleate measured with a dropping mercury electrode (DME) was studied and the results are shown in Table II-7.

Table II-7 : Effect of maleic acid on the peak height of chlorpheniramine maleate (11 ppm) in 0.2 M sulphuric acid measured with a DME at -0.775 V vs Ag/AgCl electrode

Maleic acid (ppm)	Peak height (μA)
0.00	1.30
0.24	1.50
0.44	1.95
6.72	3.20
12.20	4.40
24.40	8.25

The results show that maleic acid had a pronounced effect on the peak height of chlorpheniramine maleate. It is expected that the reduction wave of chlorpheniramine maleate may be affected by other ingredient with similar structure such as brompheniramine maleate.

The aim of the following work was to study the behaviour of chlorpheniramine maleate at a glassy carbon electrode with the hope to devise a procedure to determine this compound with improved selectivity.

The advantage of using a glassy carbon electrode over a dropping mercury electrode is that much more positive electrode potentials can be used without fear that the electrode itself is being oxidized. The pulse technique offers a significant advantage in that they can be used with electrodes other than the dropping mercury electrode while still retaining all the advantages of DME polarography [22]. The differential pulse mode was used because of its high sensitivity.

INSTRUMENTATION

A Princeton Applied Research Corporation (PAR) mode 174 A polarographic analyzer was used with a glassy carbon electrode and a silver-silver chloride electrode as reference electrode and a platinum wire as counter electrode.

REAGENTS

All reagents used were of analytical grade. A stock Britton Robinson buffer solution composed of boric acid, phosphoric acid and acetic acid (all 0.04 M) was prepared. Buffer solutions of different pH's were then prepared by addition of the appropriate amounts of 0.2 M sodium hydroxide to this stock solution to give the required pH.

PROCEDURE

An aliquot (10 ml) of the supporting electrolyte (Britton-Robinson buffer) was transferred by a pipette into the polarographic cell, and a spike containing a small measured value of chlorpheniramine maleate was then added. The peak height for the signal was measured at +0.655 V vs the Ag/AgCl electrode (B.R. buffer of pH 8.69) with the following instrument settings :

PAR 174 A Polarographic analyzer

Mode	Differential Pulse
Display direction	-ve
Initial potential	0.00 V
Current range	varying
Scan rate	5 mV/s
Scan direction	+ ve
Modulation amplitude	50 mV
Low pass filter	OFF

The tablet(s) was grounded and about 0.2 g of the powder was

accurately weighed and transferred to a 100 ml volumetric flask and the flask was shaken and diluted to the mark with the buffer. A suitable amount of the suspension was transferred to the cell. The amount of chlorpheniramine maleate in sample was determined by the standard addition calibration.

RESULTS AND DISCUSSION

(1) Effect of pH

The effect of pH on the peak of chlorpheniramine maleate was investigated by recording the current-voltage curves of the compound (20 ppm) in Britton Robinson buffer at various pH's using a glassy carbon electrode. The results are shown in Table II-8.

Table II-8 : Effect of pH on the peak potential and peak height of chlorpheniramine maleate (20 ppm), the scan rate and modulation amplitude being 5 mv/sec and 50 mV, respectively

pH	Potential (V)	Peak height (μA)
6.80	0.805	3.40
7.24	0.750	4.10
7.96	0.700	4.40
8.69	0.655	4.60
9.15	0.625	4.15
9.62	0.615	3.80
10.38	0.615	3.25
11.20	0.605	3.10

Chlorpheniramine maleate was observed to produce a well-defined peak in the pH range of 7-11. The peak potential was shifted to less positive potentials as the pH was increased.

The plot of peak potential vs pH (Figure II-3) shows two linear portions, and the break at pH 9.3 corresponds to the pKa value of the quaternary ammonium group of chlorpheniramine maleate [23], which compares well with the literature value of 9.2. [24].

As the pH of the supporting electrolyte is increased (see Table II-8), the peak height increased initially and then decreased after pH 8.69, which was then used for subsequent measurements because of the higher sensitivity obtainable at this pH.

(2) Optimization of Parameters of the Polarographic Analyzer

In differential pulse voltammetric determination using a glassy carbon electrode, the signal of the analyte will depend on the such parameters of the instrument as the scan rate and the modulation amplitude. These parameters were optimised as done previously.

The optimum scan rate and modulation amplitude in differential pulse voltammetric method were found to be 5 mV/s and 50 mV, respectively. The relevant data are shown in Table II-9 and II-10, respectively. It was noted that the modulation amplitude of 50 mV was chosen instead of 100 mV because of the

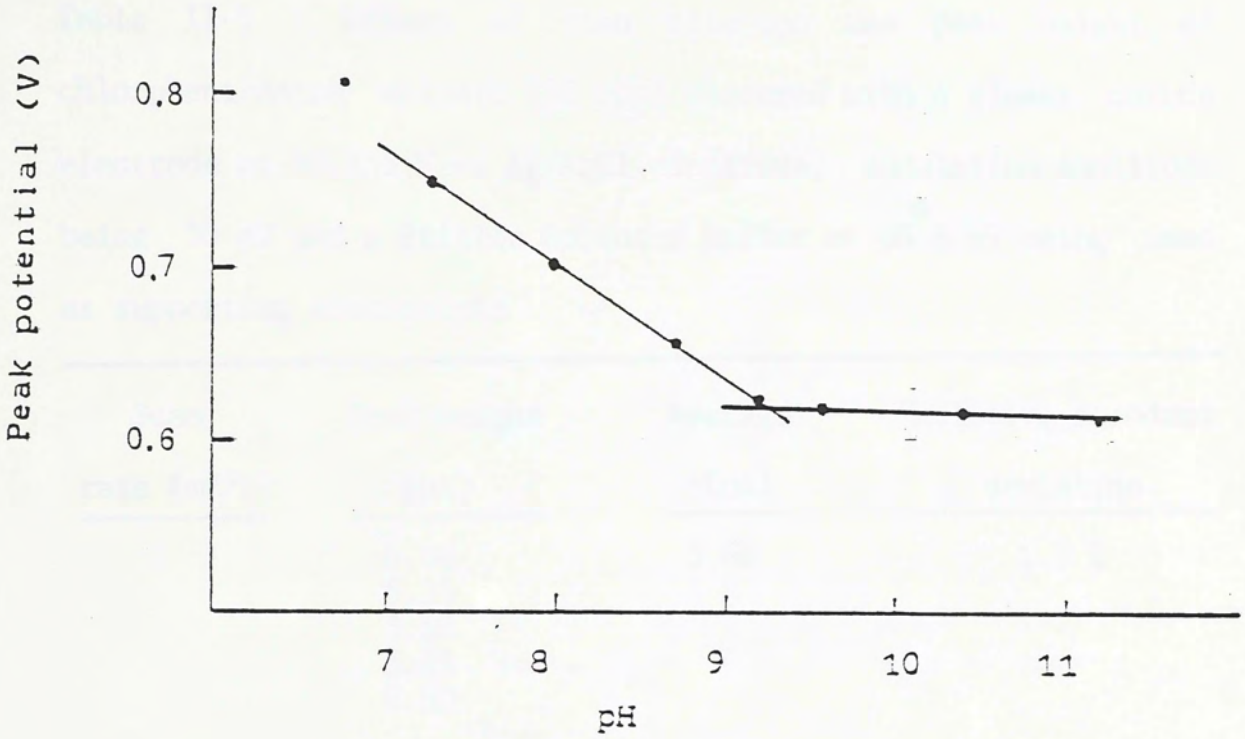


Figure II-3 : Effect of pH on peak potential of chlorpheniramine maleate measured with a glassy carbon electrode vs a Ag/AgCl electrode

better peak shape obtainable at 50 mV. The peak potential of chlorpheniramine maleate was +0.655 V vs Ag/AgCl electrode and a typical curve was shown in Figure II-4.

Table II-9 : Effect of scan rate on the peak height of chlorpheniramine maleate (20 ppm) measured with a glassy carbon electrode at +0.655 V vs Ag/AgCl electrode, modulation amplitude being 50 mV and a Britton Robinson Buffer of pH 8.69 being used as supporting electrolyte

Scan rate (mV/s)	Peak height (μA)	Average (μA)	Relative standard deviation
2	5.40 5.35 5.50 5.55 5.50	5.46	1.5 %
5	4.55 4.50 4.65 4.65 4.65	4.60	1.5 %
10	2.25 2.40 2.45 2.25 2.40	2.35	4.0 %

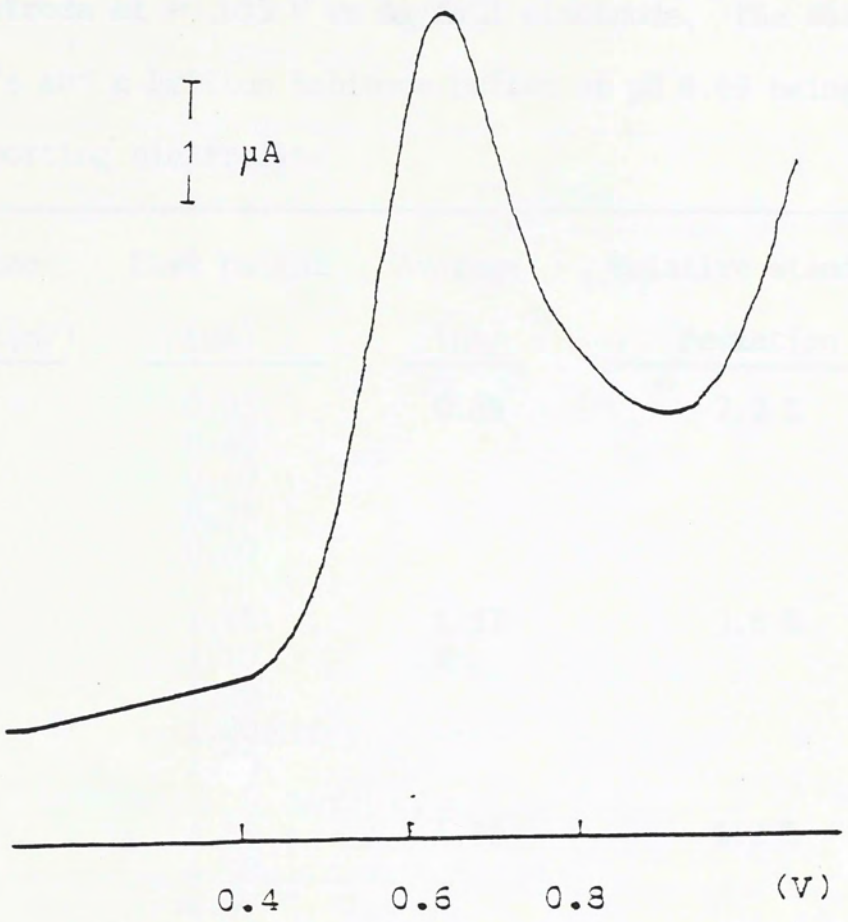


Figure II-4 : Signal of chlorpheniramine maleate (20 ppm) measured with a glassy carbon electrode

Table II-10 : Effect of modulation amplitude on the peak height of chlorpheniramine maleate (20 ppm) measured with a glassy carbon electrode at +0.655 V vs Ag/AgCl electrode, the scan rate being 5 mV/s and a Britton Robinson buffer of pH 8.69 being used as the supporting electrolyte

Modulation amplitude (mV)	Peak height (μA)	Average (μA)	Relative standard deviation
10	0.35 0.40 0.40 0.35 0.40	0.38	7.2 %
25	1.15 1.10 1.20 1.20 1.20	1.17	3.8 %
50	4.55 4.50 4.60 4.65 4.60	4.58	1.2 %
100	8.05 8.10 8.25 8.25 8.10	8.15	1.1 %

(3) Precision and Calibration Curve

The precision of the differential pulse voltammetric method using a glassy carbon electrode was studied, and the relative standard deviation for five replicate determinations of a 10 ppm chlorpheniramine maleate solution was found to be 2.68 %. The relevant data are shown in Table II-11.

Table II-11 : Test of precision for the determination of chlorpheniramine maleate (10 ppm) by differential pulse voltammetry using a glassy carbon electrode

Trial number	Peak height (μA)
1	1.55
2	1.55
3	1.60
4	1.60
5	1.50

Average : 1.56 μA

Standard deviation : 0.0418 μA

Relative standard deviation : 2.68 %

A typical calibration graph is shown in Figure II-5, and the relevant data for this graph are shown in Table II-12.

Figure II-5 shows that the calibration graph was linear for chlorpheniramine maleate concentration range of 2-19 ppm. Thus, the voltammetric method is suited for the determination of chlorpheniramine maleate in cough drugs.

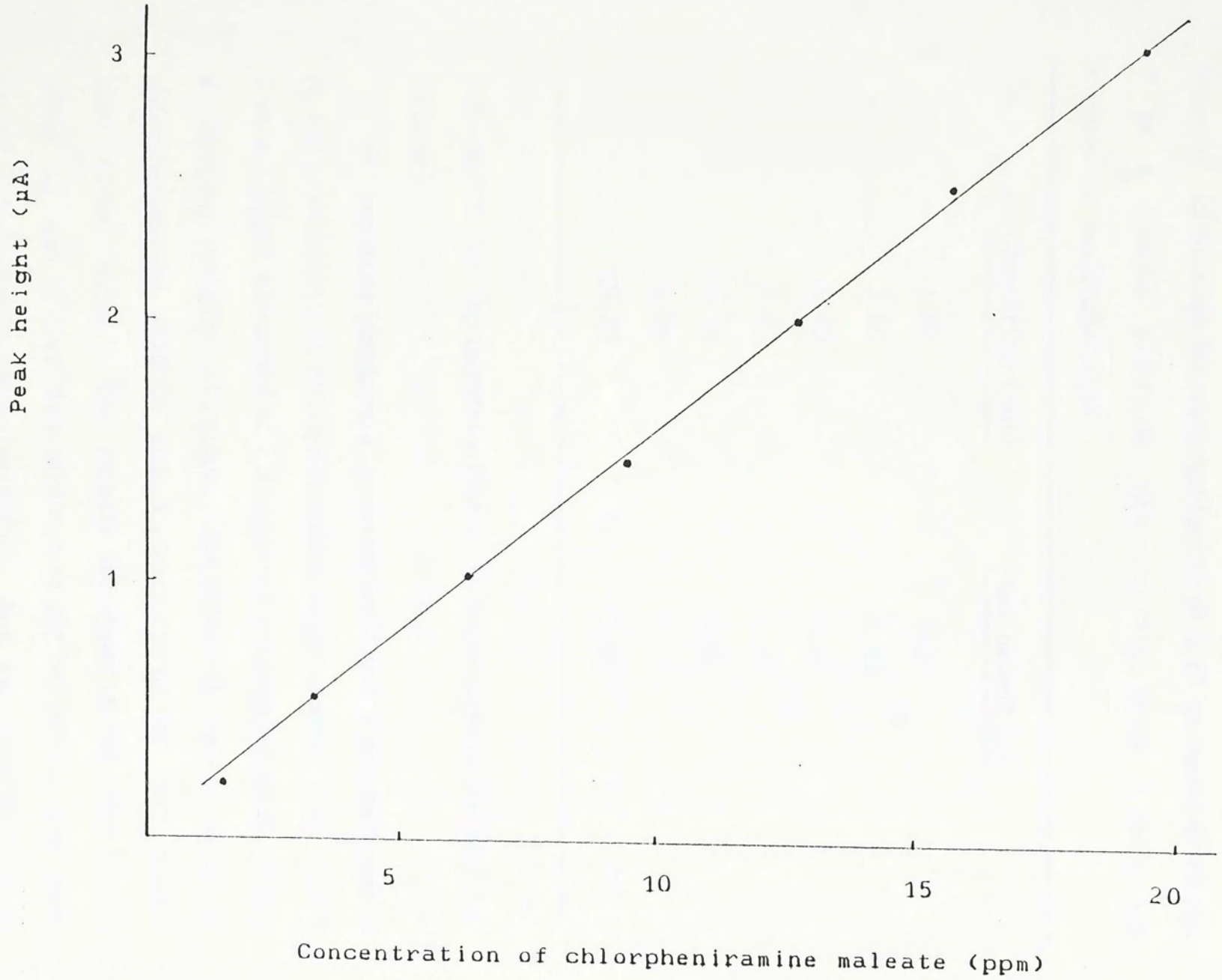


Figure II-5 : Calibration curve of chlorpheniramine maleate

Table II-12 : Data for the calibration graph of chlorpheniramine maleate in Britton Robinson buffer of pH 8.69 measured at +0.655 V vs a Ag/AgCl electrode, the scan rate being 5 mV/s and modulation amplitude 50 mV

Concentration (ppm)	Peak height (μA)
1.60	0.20
3.20	0.55
6.40	1.00
9.60	1.45
12.80	2.00
16.00	2.60
19.20	3.00

(4) Results for the Determination of Chlorpheniramine Maleate in Tablets

As reported previously , maleic acid had a profound effect on the peak height of chlorpheniramine maleate measured at -0.775 V vs a Ag/AgCl electrode by differential pulse polarography using a dropping mercury electrode. The effect of maleic acid of chlorpheniramine maleate when a glassy carbon was used needed also to be studied. The results are shown in the Table II-13, where it can be seen that maleic acid did not affect the peak height of chlorpheniramine maleate. Thus the chlorpheniramine group itself and not the maleate ion was expected to be involved in the electrochemical process at +0.665 V vs a Ag/AgCl electrode.

Table II-13 : Effect of maleic acid on the peak height of 13.5 ppm chlorpheniramine maleate measured by differential pulse voltammetry using a glassy carbon electrode at +0.655 V vs a Ag/AgCl electrode

Maleic acid (ppm)	Peak height (μA)
0.0	2.30
11.6	2.35
34.8	2.30
46.4	2.35
58.0	2.40

The contents of chlorpheniramine maleate in several tablets were then determined by the differential pulse voltammetry using standard addition calibration. The results are summarised in Table II-14 along with those obtained from British Pharmacopoeia standard method.

Table II-14 : Determination of chlorpheniramine maleate in tablets by differential pulse voltammetry using a glassy carbon electrode.

Sample	Chlorpheniramine maleate content (μA/tablet)		
	Proposed voltammetric method	Proposed polarographic method	B.P. method
1	1.96		
	1.85		
	average : 1.91 (4.1 %)*	1.94	2.02
2	1.78		
	1.89		
	average : 1.84 (4.2 %)*	1.85	1.92
3	3.81		
	3.59		
	average : 3.70 (4.2 %)*	3.72	3.88

* relative standard deviation quoted in parentheses

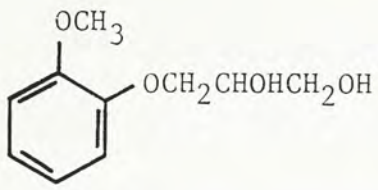
The results obtained using the proposed voltammetric method are comparable to those obtained from the B. P. standard method and the relative errors were less than 6 %. It is noted that the proposed method requires only direct dissolution of tablets into the supporting electrolyte, and the procedure is much simpler

than the B. P. method.

Results of the chlorpheniramine maleate content in tablets obtained by the differential pulse polarographic method using a DME as reported in Table II-6 are also included in Table II-14 for comparision. It can be seen that the results obtained using both differential pulse polarographic and differential pulse voltammetric methods agree well in the determination of chlorpheniramine maleate tablets. Thus, both methods can be used. However, in mixtures where maleate or maleic acid is present, the voltammetric method using a glassy carbon electrode should be used.

Chapter III : THE DETERMINATION OF GUAIFENESIN

Guaifenesin is a common expectorant in cough mixtures and has also been used as a muscle relaxant during surgery, and its structure is shown below :



The structure of guaifenesin

The amount of guaifenesin in a mixture can be assayed by a pharmacopoeial method [13], and it has also been determined in pharmaceutical preparation by gas liquid chromatography [24] [25].

Guaifenesin has not, so far, been measured by any voltammetric method. The object of the present work is to study the voltammetric behaviour of guaifenesin using the differential pulse technique and with the glassy carbon electrode as the working electrode.

INSTRUMENTATON

A PAR mode 174 A polarographic analyzer was used. A glassy carbon electrode, a silver-silver chloride electrode and a platinum wire were used as the working electrode, reference electrode and the counter electrode, respectively.

REAGENTS

All reagents used were of analytical grade. A stock Britton Robinson buffer solution composed of boric acid, phosphoric acid and acetic acid (all 0.04 M) was prepared. Buffer solutions of different pH's were then prepared by the addition of appropriate amount of 0.2 M sodium hydroxide to the stock solution to the required pH.

PROCEDURE

An aliquot (10 ml) of the supporting electrolyte was transferred into the polarographic cell, and a spike containing a measured value of guaifenesin was then added. The peak height for the signal was measured at + 1.055 V vs the Ag/AgCl electrode by using the tangent-fit method, and with the following instrument settings :

PAR 174 A Polarographic Analyzer

Mode	Differential pulse
Display direction	-ve
Initial potential	+ 0.5 V
Current range	varying
Scan rate	5 mV/s
Scan direction	+ ve
Modulation amplitude	50 mV

An aliquot (5 ml) of the mixture was transferred into a 250 ml volumetric flask and diluted to the mark with the Britton Robinson buffer of pH 3.29. One ml of the diluted sample

solution was transferred into the polarographic cell and 9 ml of the supporting electrolyte was added. The peak height was measured at + 1.105 V vs Ag/AgCl electrode.

RESULTS AND DISCUSSION

(1) Effect of pH

Results on the effect of pH on the peak of guaifenesin are shown in Table III-1. The Britton Robinson buffer of various pH's were used as the supporting electrolytes.

Table III-1 : Effect of pH on the peak potential measured vs a Ag/AgCl electrode and the peak height of guaifenesin (3.7 ppm), the scan rate and modulation amplitude being 5 mV/s and 50 mV, respectively

pH	Peak potential (V)	Peak height (μA)
3.29	+ 1.105	3.00
4.10	+ 1.105	2.75
4.56	+ 1.105	2.67
5.02	+ 1.110	2.15
5.72	+ 1.105	1.80
6.37	+ 1.105	1.05
6.80	+ 1.085	1.20
7.24	+ 1.085	1.15
7.96	+ 1.070	1.05
8.69	+ 1.055	0.70
9.15	+ 1.045	0.70

Guaifenesin was found to produce a well-defined wave in the working pH range of 3-9, and a typical curve for guaifenesin in Britton Robinson buffer of pH 8.69 is shown in Figure III-1. Results in Table III-1 show that the peak height decreased with increasing pH.

The plot of peak potential against pH (Figure III-1) may be divided into two linear portions. There is a break at about pH 6.4.

(2) Optimization of Parameters of the Polarographic Analyzer

The optimum scan rate was found to be 5 mV/s and the modulation amplitude to be 50 mV. The relevant data are shown in Tables III-2 and III-3, respectively.

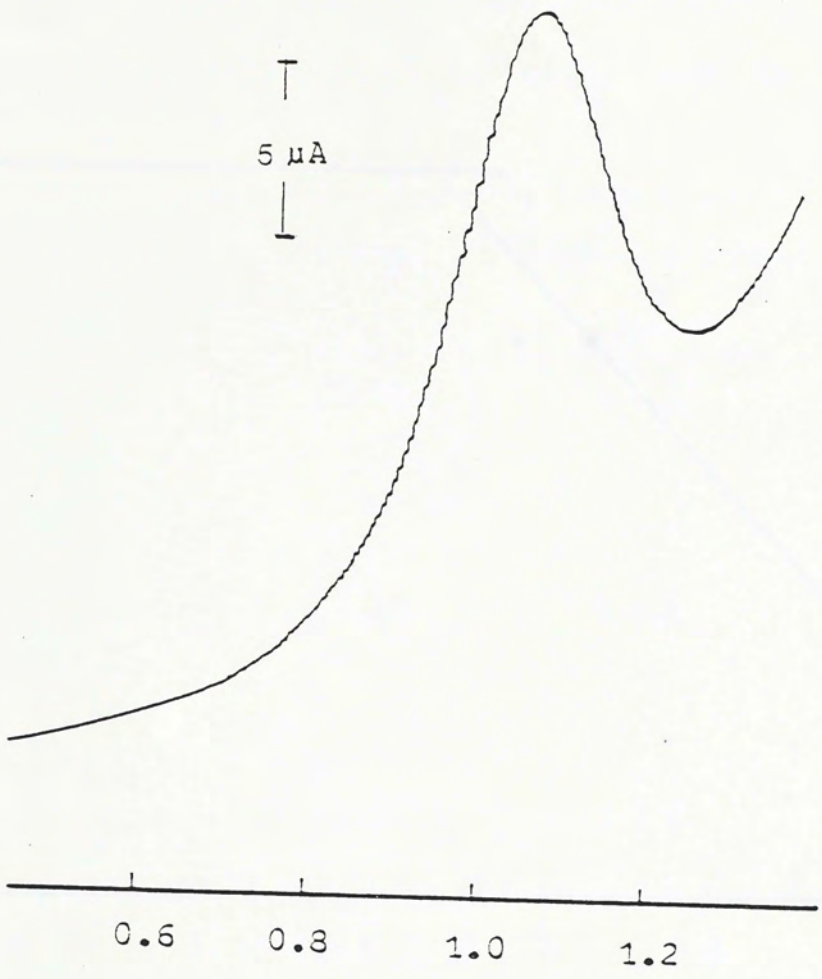


Figure III-1 : Signal of guaifenesin measured with a glassy carbon electrode and a Brittton Robinson buffer (pH 3.29) as supporting electrolyte

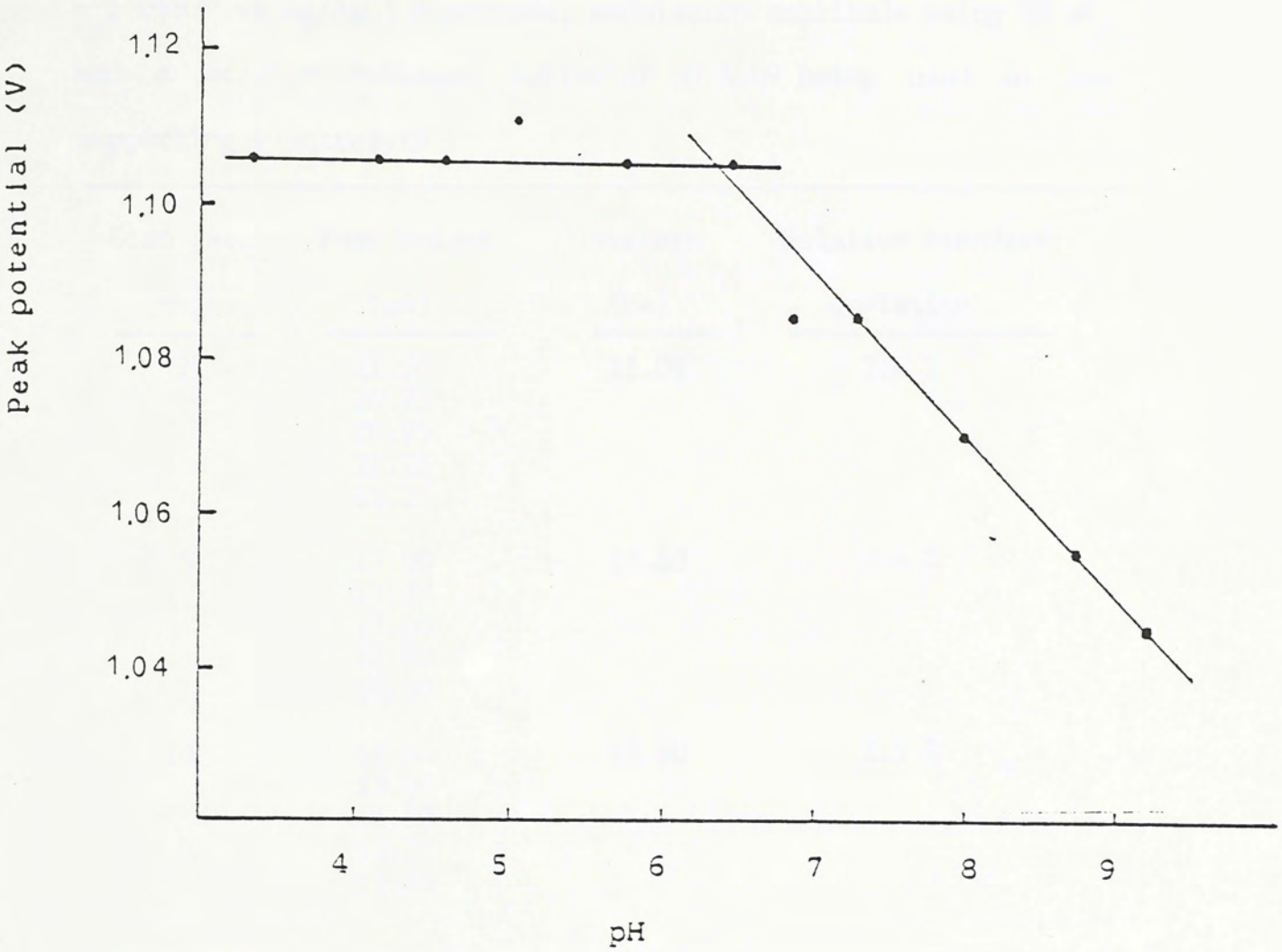


Figure III-2 : Effect of pH on the peak potential of guaifenesin measured with a glassy carbon vs a Ag/AgCl electrode

Table III-2 : Effect of scan rate on the peak height of guaifenesin (59 ppm) measured with a glassy carbon electrode at + 1.055 V vs Ag/AgCl electrode, modulation amplitude being 50 mV, and a Britton Robinson buffer of pH 8.69 being used as the supporting electrolyte

Scan rate (mV/s)	Peak height (µA)	Average (µA)	Relative standard deviation
2	21.50 20.25 20.75 21.25 21.25	21.00	2.4 %
5	17.00 17.25 17.00 17.50 18.00	17.35	2.4 %
10	12.50 13.00 12.50 13.25 12.75	12.80	2.5 %

Table III-3 : Effect of modulation amplitude on the peak height of guaifenesin (59 ppm) measured with a glassy carbon electrode at + 1.055 V vs Ag/AgCl electrode, the scan rate being 5 mV/s and a Britton Robinson buffer of pH 8.69 being used as the supporting electrolyte

Modulation amplitude (mV)	Peak height (μA)	Average (μA)	Relative standard deviation
10	2.25 2.50 2.50 2.00 2.25	2.30	9.1 %
25	6.50 6.75 6.75 6.50 6.50	6.60	2.1 %
50	17.00 17.25 17.00 17.50 17.50	17.25	1.4 %
100	39.25 39.00 39.50 39.00 39.75	39.30	0.8 %

(3) Precision and Calibration Curve

The relative standard deviation for five replicate determinations of a 15 ppm guaifenesin solution using the proposed method was calculated to be 1.29 %. The relevant data are shown in Table III-4.

Table III-4 : Test of precision for the determination of guaifenesin (15 ppm) by differential pulse voltammetry using a glassy carbon electrode.

Trial number	Peak height (μA)
1	5.75
2	5.75
3	5.80
4	5.75
5	5.65
Average :	5.76 μA
Standard deviation :	0.0742 μA
Relative standard deviation :	1.29 %

A typical calibration graph is illustrated in Figure III-3, and the relevant data for the graph are shown in Table III-5. The graph is linear in the range of 2-30 ppm guaifenesin at pH 3.29.

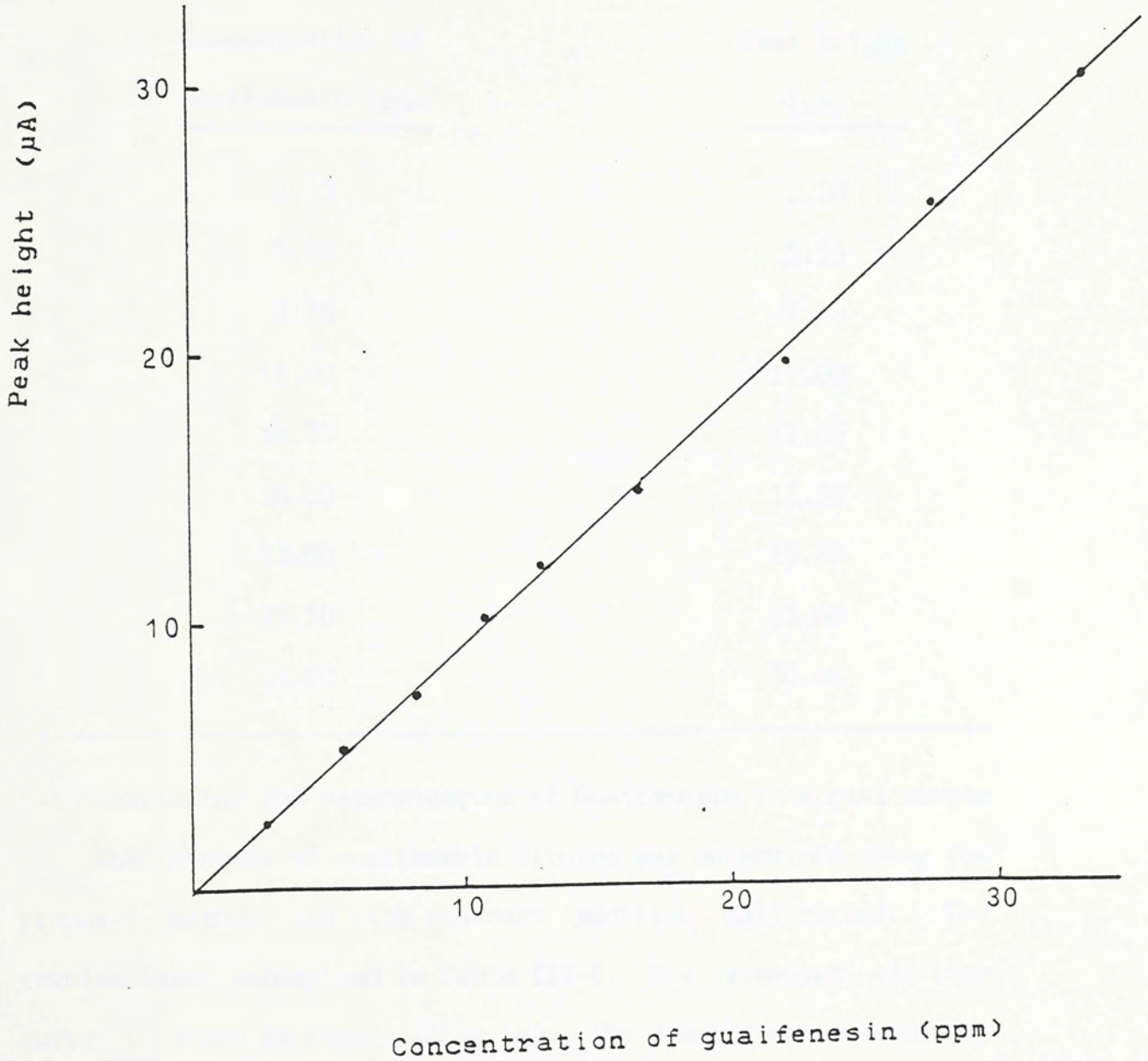


Figure III-3 : Calibration curve of guaifenesin

Table III-5 : Data for the calibration graph of guaifenesin in Britton Robinson buffer of pH 3.29 measured at +1.105 V vs a Ag/AgCl electrode, the scan rate being 5 mV/s and modulation amplitude 50 mV

Concentration of Guaifenesin (ppm)	Peak height (μA)
2.75	2.50
5.50	5.25
8.25	7.40
11.00	10.00
12.75	12.05
16.50	15.50
22.00	19.80
27.50	25.60
33.00	30.40

(4) Results for the Determination of Guaifenesin in a real sample

The content of guaifenesin linctus was determined using the proposed method and with standard addition calibration. The results are summarised in Table III-6. The standard addition curve is shown in Figure III-4 (a). The results were checked by standard method of United States Pharmacopoiea National Formulary (USPNF) for comparison.

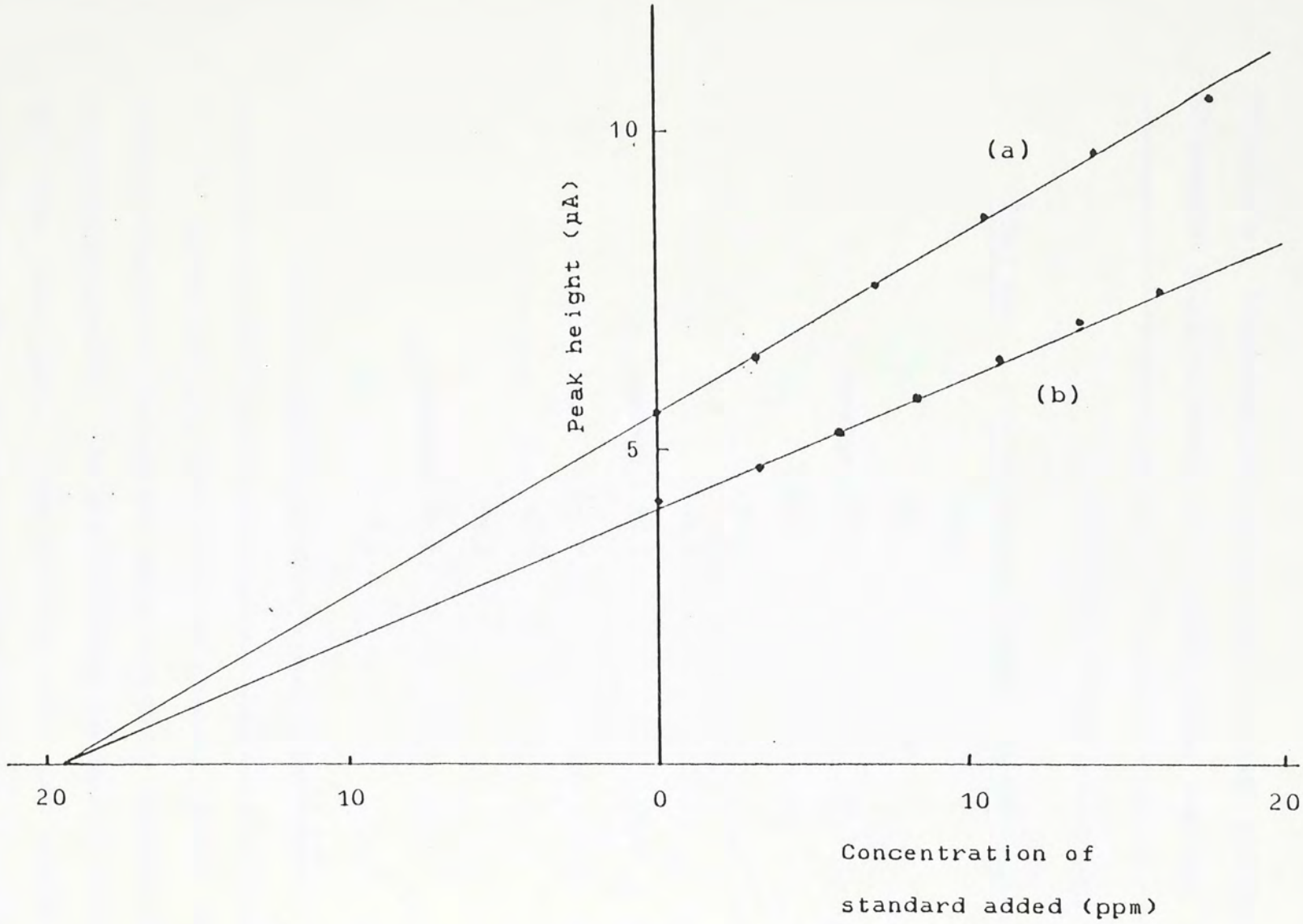


Figure III-4 : Standard addition curve for the determination guaifenesin (a) without absolute ethanol, (b) with 11.8 % absolute ethanol

Table III-6 : Determination of guaifenesin in a cough linctus by differential pulse voltammetry with a glassy carbon electrode

	Guaifenesin in a cough linctus (mg)	
Trial no.	by voltammetric method *	by USPNF method
1	97.2	
	95.4	
	average : 96.3	99.5
	(1.3 %)	
2	96.5	
	94.9	
	average : 95.7	98.0
	(1.2 %)	
3	97.0	
	99.2	
	average : 98.1	101.1
	(1.6 %)	

* relative standard deviation quoted in parentheses

It can be seen that there is a close agreement between the results obtained by the proposed method with those obtained by the established method. The precision using the proposed method was also very good. When compared with the standard pharmacopoeia method, the proposed voltammetric method is simpler and faster because it does not require any extraction.

(5) Effect of Pretreatment of the Glassy Carbon Electrode

A home - made glassy - carbon electrode (GCE) was used in this study, and it was made of a disc of glassy carbon, which was sealed to a glass tube. The electrical contact was made by means of a small amount of mercury inside the tube and a platinum wire.

A disadvantage of solid electrodes is that the reproducibility of the current - voltage behaviour cannot be as good as that of the dropping mercury electrode, which continuously provides a new and fresh electrode surface. The behaviour of the solid electrodes is often affected by the adsorption or deposition on its surface. As a result, it is evident that solid electrode need some kind of pretreatment in order to obtain reproducible results.

There are many different procedures described in the literatures, including polishing the surface with alumina or chromium (III) oxide suspension [27], rinsing with a mixture of sulfuric acid and nitric acid [28], simple cathodic cleansing [29] and by polarization cycles [30] [31].

Throughout this study, two kinds of electrode pre-treatment procedures were adopted. The surface of the glassy carbon electrode was polished daily prior to each series of measurements with a polishing cloth wet with an abrasive slurry of alumina.

Prior to each measurement, the potential of the electrode was held at - 1.2 V for a few minutes. This procedure might remove the adsorbed films on the electrode surface and restore the reproducibility of the currents (peak height) and even the peak potentials.

In the following table, the effect of holding at - 1.2 V for five minutes on the reproducibility of the peak height of guaifenesin is illustrated. The data show that the reproducibility of the peak height can be restored after cathodic cleansing. On the other hand, the peak height decreased significantly after each repeat scan of the sample without holding at negative potential.

Table III-7 : The effect of cathodic cleansing on the reproducibility of peak height of 16.5 ppm guaifenesin in pH 3.29 Britton - Robinson buffer supporting electrolyte

	Peak height (μA)	
Trial	with holding at - 1.2 V	without holding at - 1.2 V
(1)	10.0	10.0
(2)	9.8	9.4
(3)	9.8	9.0
(4)	10.0	8.6

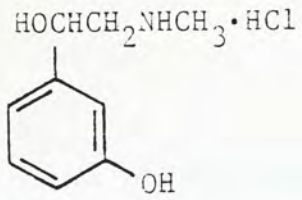
The problem of adsorption was more serious when several active ingredients were present and cathodic cleansing of glassy

carbon electrode provided little improvement.

When ethanol was added to the buffer, the reproducibility of the signal was improved. However, since the presence of ethanol would suppress the peak height and hence the sensitivity, the amount of solvent used must be controlled. It was found that the presence of 11.8 % (v/v) of absolute ethanol did not affect the results for the determination of guaifenesin in a real sample (see Figure III-4).

Chapter IV : THE DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE

Phenylephrine hydrochloride is a decongestant in cough - cold mixtures, and its structure is shown below :



The structure of phenylephrine hydrochloride

Phenylephrine hydrochloride can be assayed by the iodometric method of the United States Pharmacopoiea. However, it has also been analysed by colorimetric methods [32] [33].

Phenylephrine hydrochloride has also not been measured by any voltammetric method. The voltammetric behaviour of phenylephrine hydrochloride was thus studied using the differential pulse technique, and with the glassy carbon electrode as the working electrode.

INSTRUMENTATION

A Princeton Applied Research Corporation mode 364 polarographic analyzer was used with the glassy carbon electrode. An Saturated Calomel Electrode was used as the reference electrode, and a platinum wire as counter electrode.

REAGENTS

All reagents used were of analytical grade. A stock Britton-Robinson buffer was prepared and buffers of different pH were obtained by the addition of appropriate amount sodium hydroxide (0.2 M).

PROCEDURE

For each measurement, 17 ml of the supporting electrolyte (Britton Robinson buffer of pH 8.69) was transferred into the polarographic cell and a measured amount of standard phenylephrine solution was then added. The peak height of the signal was measured at + 0.560 V versus Saturated Calomel Electrode, and with the following instrument settings :

PAR 364 Polarographic analyzer

Mode	Differential pulse
Initial potential	+ 0.00 V
Scan direction	+ ve
Current range	varying
Scan rate	5 mV/s
Pulse amplitude	50 mV

A synthetic tablet was prepared by mixing phenyephrine hydrochloride with starch. About 0.2 g of the powder was accurately weighed and transferred to a 100 ml-volumetric flask and Britton Robinson buffer (pH 8.69) was added. The flask was shaken and its content was diluted to the mark with the buffer.

A portion of the suspension was transferred to the cell. The amount of phenylephrine hydrochloride present in the sample was determined by the standard - addition calibration.

RESULTS AND DISCUSSION

(1) Effect of pH

Results on the effect of pH on the peak of phenylephrine hydrochloride are shown in Table IV-1. The Britton Robinson buffer of various pH's were used as the supporting electrolyte.

Table IV-1 : Effect of pH on the peak height and peak potential of phenylephrine hydrochloride (18.4 ppm), the scan rate and modulation amplitude being 5 mV/s and 50 mV, respectively

pH	Peak potential (V)	Peak height (μA)
3.29	0.915	9.75
4.10	0.880	10.50
4.56	0.850	10.75
5.72	0.755	13.25
6.80	0.685	16.50
7.96	0.585	21.50
8.69	0.560	22.50
9.15	0.545	20.75
9.62	0.530	15.75

It can be seen that the peak height increased with pH until 8.69 was reached, and after that, the peak height decreased with pH. Thus, pH 8.69 was utilised in the subsequent measurements.

The plot of peak potential vs pH is shown in Figure IV-1, where the graph is found to be consisted of two linear portions in the pH range of 3.3 to 9.6, and the break is at about pH 7.9.

(2) Optimisation of Parameter of the Polarographic Analyzer

The optimum scan rate and modulation amplitude were chosen to be 5 mV/s and 50 mV, respectively. The relevant data are shown in Tables IV-2 and IV-3.

A typical wave of phenylephrine hydrochloride is shown in Figure IV-2.

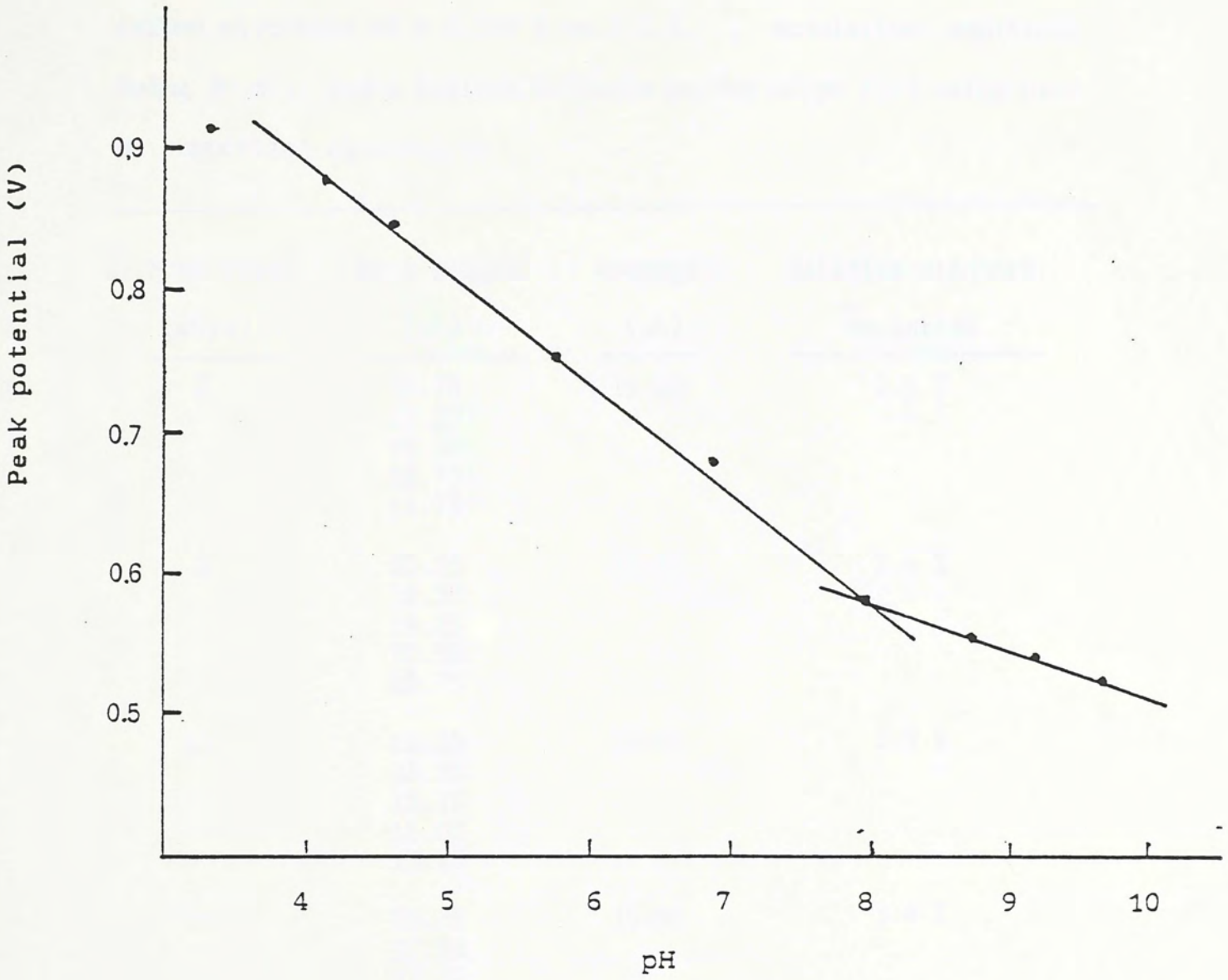


Figure IV-1 : Effect of pH on the peak potential of phenylephrine hydrochloride measured with a glassy carbon electrode vs saturated calomel electrode

Table IV-2 : Effect of scan rate on the peak height of phenylephrine hydrochloride (19.4 ppm) measured with a glassy carbon electrode at + 0.560 V vs S.C.E. , modulation amplitude being 50 mV, and a Britton Robinson buffer of pH 8.69 being used as supporting electrolyte

Scan rate (mV/s)	Peak height (µA)	Average (µA)	Relative standard deviation
2	19.75 19.25 18.50 18.75 18.75	19.00	2.6 %
5	20.25 19.50 19.25 20.50 19.75	19.85	2.6 %
10	16.25 15.50 15.25 16.00 15.25	15.65	2.9 %
20	16.25 17.00 17.50 16.50 17.50	16.95	3.4 %

Table IV-3 : Effect of modulation amplitude on the peak height of phenylephrine hydrochloride (19.4 ppm) measured with a glassy carbon electrode at + 0.560 V vs S.C.E. , the scan rate being 5 mV/s and B.R. buffer (pH 8.69) being used as the supporting electrolyte

Modulation amplitude (mV)	Peak height (μA)	Average (μA)	Relative standard deviation
25	3.50 3.75 3.50 3.75 3.75	3.65	3.8 %
50	20.25 19.50 20.50 19.25 19.75	19.85	2.6 %
100	24.50 24.00 25.50 25.50 25.25	24.95	2.7 %

(3) Precision and calibration curve

The relative standard deviation for five replication determination of a 6.5 ppm standard phenylephrine hydrochloride solution using the proposed method was found to be 1.5 % and is quite good. The relevant data are shown in Table IV-4.

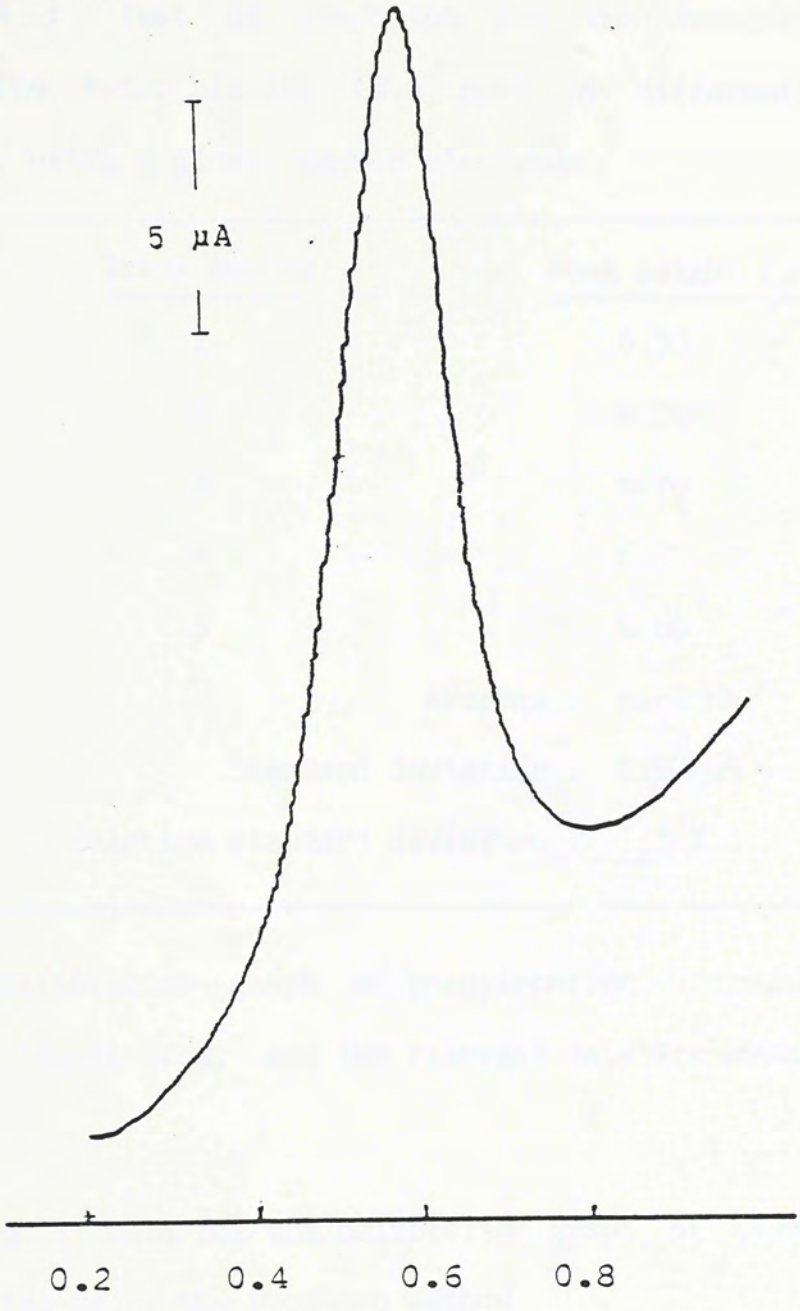


Figure IV-2 : Signal of phenylephrine hydrochloride with a glassy carbon electrode vs at a scan rate of 5 mV/s and modulation amplitude of 50 mV

Table IV-4 : Test of precision for the determination of phenylephrine hydrochloride (6.5 ppm) by differential pulse voltammetry using a glassy carbon electrode

Trial number	Peak height (μA)
1	6.50
2	6.50
3	6.70
4	6.70
5	6.60
Average :	6.60 μA
Standard deviation :	0.10 μA
Relative standard deviation :	1.5 %

The calibration graph of phenylephrine hydrochloride is plotted in Figure IV-3, and the relevant data are shown in Table IV-5.

Table IV-5 : Data for the calibration graph of phenylephrine hydrochloride using the proposed method

Concentration (ppm)	Peak height (μA)
1.83	1.5
3.64	3.4
5.44	5.5
7.22	7.4
8.98	9.1
10.72	10.6
12.44	12.5

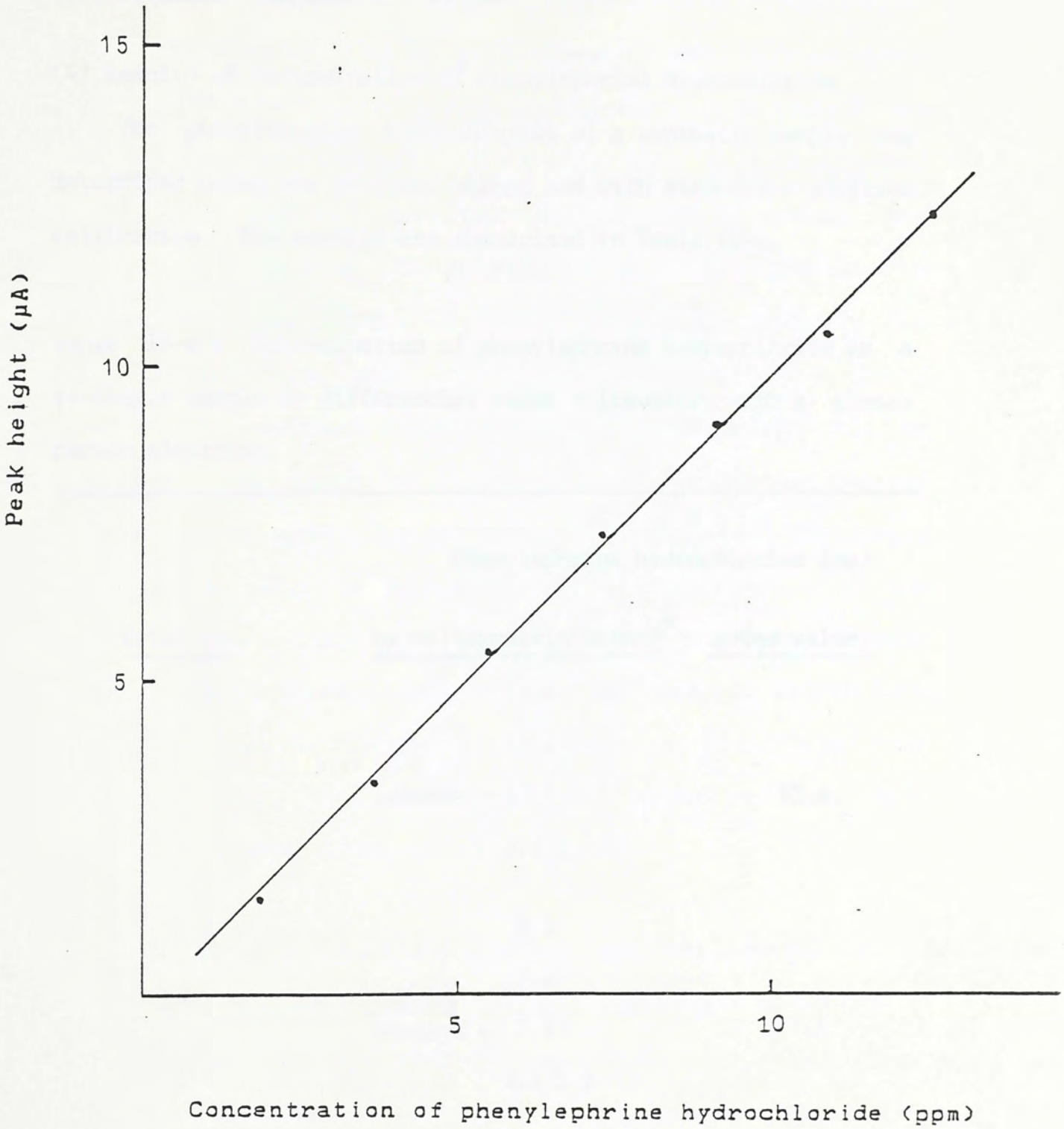


Figure IV-3 : Calibration graph for phenylephrine hydrochloride

The calibration graph was found to be linear in the concentration range of 2 - 12 ppm.

(4) Results of Determination of Phenylephrine Hydrochloride

The phenylephrine hydrochloride of a synthetic sample was determined using the proposed method and with standard - addition calibration. The results are summarised in Table IV-6.

Table IV-6 : Determination of phenylephrine hydrochloride in a synthetic sample by differential pulse voltammetry with a glassy carbon electrode

	Phenylephrine hydrochloride (mg)	
Trial no.	by voltammetric method*	added value
1	13.8	
	13.1	
	average : 13.5	12.8
	(3.7 %)	
2	8.1	
	7.6	
	average : 7.9	7.5
	(4.5 %)	

* relative standard deviation quoted in parentheses

CONCLUSION

Differential pulse voltammetric method for the determination of chlorpheniramine maleate, guaifenesin and phenylephrine hydrochloride in pure form or in drug formulation such as tablets and linctus has been developed. The results show a close agreement with those obtained by the standard pharmacopoeial methods, and the precision is good. The proposed method involves the use of a home-made glassy carbon electrode, which is necessary for ingredients giving waves at applied potentials above + 0.4 V versus the saturated calomel electrode, where mercury will be oxidized. The supporting electrolyte is the Britton-Robinson buffer of the appropriate pH values. In addition, the sample preparation requires only dilution or dissolution with the supporting electrolyte without any further treatment, because insoluble constituents in tablets and excipients in linctus have not been observed to cause any significant interference and need not be removed. Thus the method is much simpler and more rapid than the standard method from pharmacopeia.

It is also worthy to note that the voltammetric method provides a convenient method for the determination of the pKa of the conjugate acids of amine-derived drugs.

The proposed method should also be applicable to determine other active ingredients in cough - cold drugs giving waves at applied potentials above + 0.4 V versus the saturated calomel electrode.

Chapter V : THE SIMULTANEOUS DETERMINATION OF CHLORPHENIRAMINE MALEATE, GUAIFENESIN AND PHENYLEPHRINE HYDROCHLORIDE IN COUGH MIXTURES

Mixtures used for the treatment of coughs and cold may be rather complex and contain several active ingredients. The liquid formulations may also contain preservatives, dyes, sweeteners and flavours.

It is the purpose of the present work to apply the voltammetric method developed for chlorpheniramine maleate, guaifenesin and phenylephrine hydrochloride as described in Chapters II to IV to determine these cough ingredients in mixtures simultaneously.

In this chapter, the differential pulse voltammetric method was attempted to determine the active ingredients of cough mixture simultaneously.

INSTRUMENTATION

A PAR 364 polarographic analyzer was used. A glassy carbon electrode and a saturated calomel electrode (SCE) were used as the working electrode and the reference electrode, respectively. The minimum working volume of the cell was about 17 ml.

REAGENTS

All reagents used were of analytical grade.

PROCEDURE

The polarogram of each sample was recorded with the following instrument settings :

PAR 364 polarographic analyzer

Mode	Differential pulse
Potential scan	5 mV/s
Scan direction	+ ve
Pulse amplitude	50 mV

The peak potentials at pH 8.69 of the three active ingredients under study were :

Chlorpheniramine maleate	+ 0.675 V
Guaifenesin	+ 1.055 V
Phenylephrine hydrochloride	+ 0.560 V

RESULTS & DISCUSSION

(1) The Optimum Experimental Conditions

The optimum modulation amplitude of 50 mV and scan rate of 5 mV/s chosen for each of the three ingredients under study happened to be the same. These values could thus be used for the simultaneous determination of these compounds.

However, the optimum pH values were not entirely the same. They were 8.69 for chlopheniramine maleate , 3.29 for guaifenesin and 8.69 for phenylephrine hydrochloride. The pH chosen for the

simultaneous determination was 8.69 for the obvious reason that it was the optimum pH for both chlorpheniramine maleate and phenylephrine hydrochloride, and more importantly for the reason that the peak potentials for these two compounds will be shifted to more positive values, where the background signals are high.

(2) Interference Study

In the previous chapters, relatively simple samples were analysed for the contents of the active ingredients because those samples were either tablets or linctus containing one active component only. Thus, for the determination of active ingredients in cough mixture, the effects of the excipients and the mutual interferences of the active ingredients needed to be studied.

(a) Interference from excipients

Excipients such as preservatives, dyes and flavours can be divided into two categories, namely, the voltammetric active and inactive species. The voltammetric active excipients such as methyl paraben produces signal with peak potential which may overlap with those of the active ingredients of interest. On the other hand, voltammetric inactive excipients do not show any signal.

(i) Excipients without voltammetric activity

These excipients show no signal within the range of the applied potentials. However, they may affect the peak height of

the active ingredients to various extents and may even affect the peak potentials. The criterion for an interference was the peak height varying by ± 5 % from the expected value. The effects of a number of common excipients were examined by applying the method to solutions containing chlorpheniramine (16 ppm), guaifenesin (17 ppm) and phenylephrine (13 ppm), respectively. Results of the studies are shown in Table V-1.

Table V-1 : Effect of voltammetric inactive excipients on the peak height of the active ingredients

Voltammetric inactive ingredient	Chlorpheniramine maleate (16 ppm)		Guaifenesin (17 ppm)		phenylephrine HCl (13 ppm)	
	* conc. (ppt)	dev., %	* conc. (ppt)	dev., %	* conc. (ppt)	dev., %
Ammonium chloride	0.10	- 5.3	0.42	- 4.1	0.07	- 5.9
Chloroform (in EtOH)	4	- 4.9	20	- 1.0	2	- 4.0
Ethanol	300	- 4.4	1000	- 2.7	150	- 4.9
Fructose	0.01	- 5.7	0.07	- 5.4	0.01	- 5.4
Sodium benzoate	0.03	+ 5.4	0.01	+ 3.9	0.03	+ 3.2
Sodium citrate	0.08	- 4.4	0.01	+ 4.9	0.01	- 3.5
Sucrose	0.11	+ 5.1	0.01	- 5.0	0.01	- 3.9

** shift in peak potential

* concentration of the interference added

It can be seen from the results that most of the voltammetric inactive excipients suppressed the peak heights of the ingredients under study. Fortunately, the interferences are not serious when these excipients are present at the levels shown in Table V-1. Furthermore, it was observed that the presence of absolute ethanol shifted the peak potential of guaifenesin to the more positive potential. The effect of absolute ethanol on the peak potential was studied and the results are shown graphically in Figure V-1.

The determination of guaifenesin in a real sample has been studied in the presence of 11.76 % absolute ethanol. The standard addition calibration was previously shown in Figure III-4 (b). The peak height was measured at the peak potential + 1.135 V versus Saturated Calomel Electrode. The results obtained was similar to that obtained where no absolute ethanol had been added. However, the peak heights were suppressed in the presence of ethanol.

(ii) Excipients with voltammetric activity

Our preliminary experiments showed that a number of common excipients produced well- defined peaks within the range of the applied potential of + 0.2 V to + 1.3 V versus S C E. The voltammetric active excipients and their peak potential are shown in Table V-2.

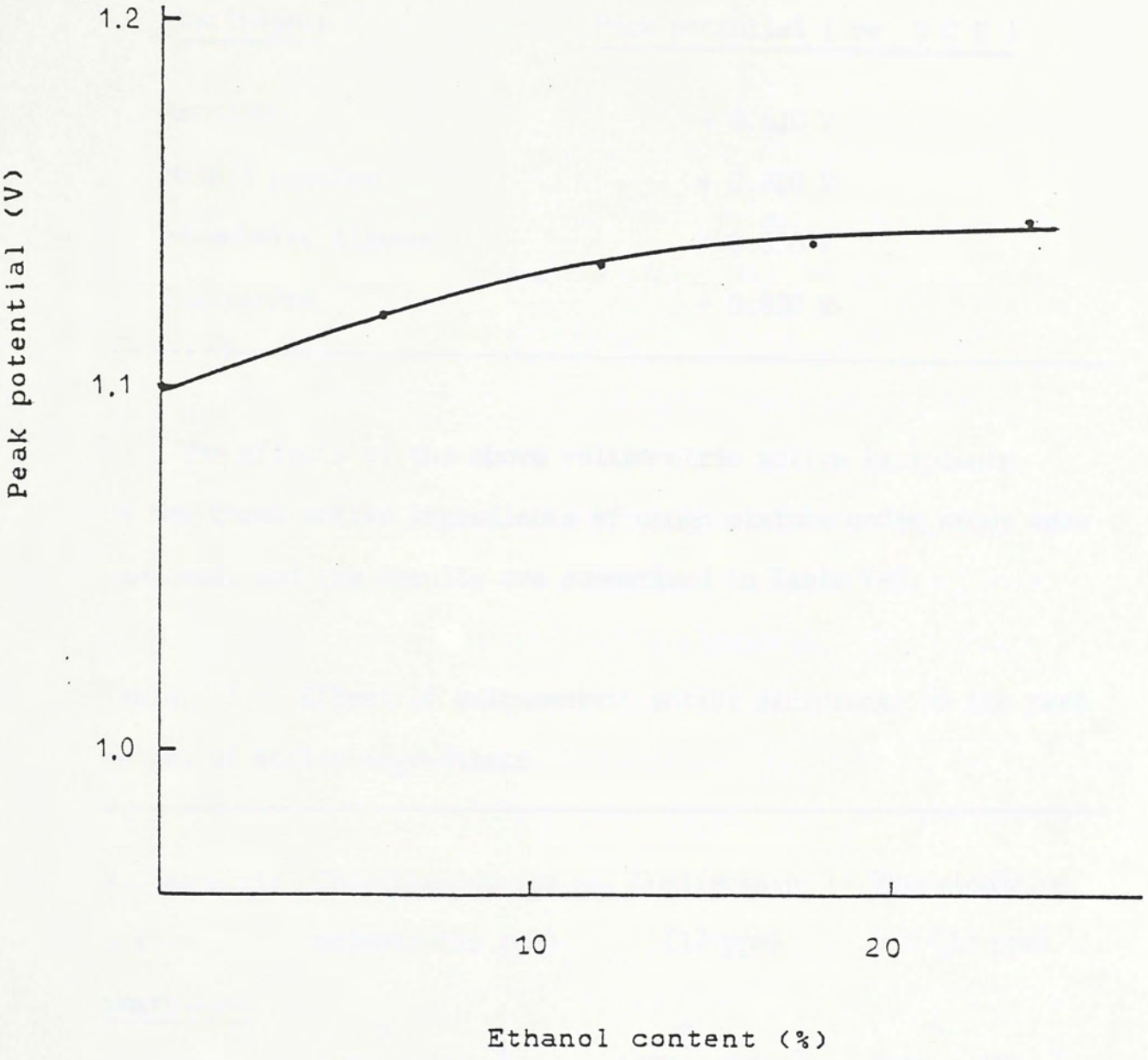


Figure V-1 : Effect of ethanol on the peak potential of guaifenesin

Table III-2 : The peak potentials of several common non - active ingredients in cough - cold mixture

Excipients	Peak potential (vs S C E)
Amaranth	+ 0.610 V
Methyl paraben	+ 0.700 V
Strawberry flavour	+ 0.550 V
Tartrazine	+ 0.830 V

The effects of the above voltammetric active excipients on the three active ingredients of cough mixture under study were examined, and the results are summarised in Table V-3.

Table V-3 : Effect of voltammetric active excipients on the peak height of active ingredients

Voltammetric active ingredient	Chlorpheniramine maleate (16 ppm)		Guaifenesin (17 ppm)		Phenylephrine HCl (13 ppm)	
	* conc. (ppm)	dev.,%	* conc. (ppm)	dev.,%	* conc. (ppm)	dev.,%
Amaranth	9.6	+ 4.7	6.8	+ 4.4	11.7	- 4.4
Methyl paraben	12.8	+ 3.8	0.17	+ 3.0	5.2	- 5.3
Strawberry flavour	50	+ 7.0	100	+ 5.4	100	- 1.0
Tartrazine	12.8	+ 0.4	6.8	+ 5.0	5.2	+ 3.2

* concentration of the interferent added

The results show that these excipients did not produce significant interferences when present at the levels under study. The reason is that although these excipients exhibit peaks at about + 0.5 V to + 0.8 V, yet the responses of those peaks are relatively low compared to those of the ingredients of interest, and the amount of such excipients in cough - cold drug are usually small so it is expected that the effects of these excipients will be insignificant, particularly when the determinations were done by the standard - addition calibration.

A recovery test was performed for the determination of chlorpheniramine maleate in the presence of 0.01 % strawberry flavour, 48 ppm tartrazine and 50.7 ppm amaranth. The standard - addition calibration graph is plotted in Figure V-2. The recovery of chlorpheniramine maleate was found to be 94.7 %. The peak height was observed to be suppressed in a mixture of these excipients, and thus large amounts of chlorpheniramine maleate were added to produce large enough peak heights for measurements. However, the linearity of the calibration graph was not affected.

(b) Interference from active ingredients

In a mixture of a number of active ingredients, these ingredients are expected to exhibit mutual interference on each other, especially when their peak potentials are close.

In this section, the effect of two pairs of active

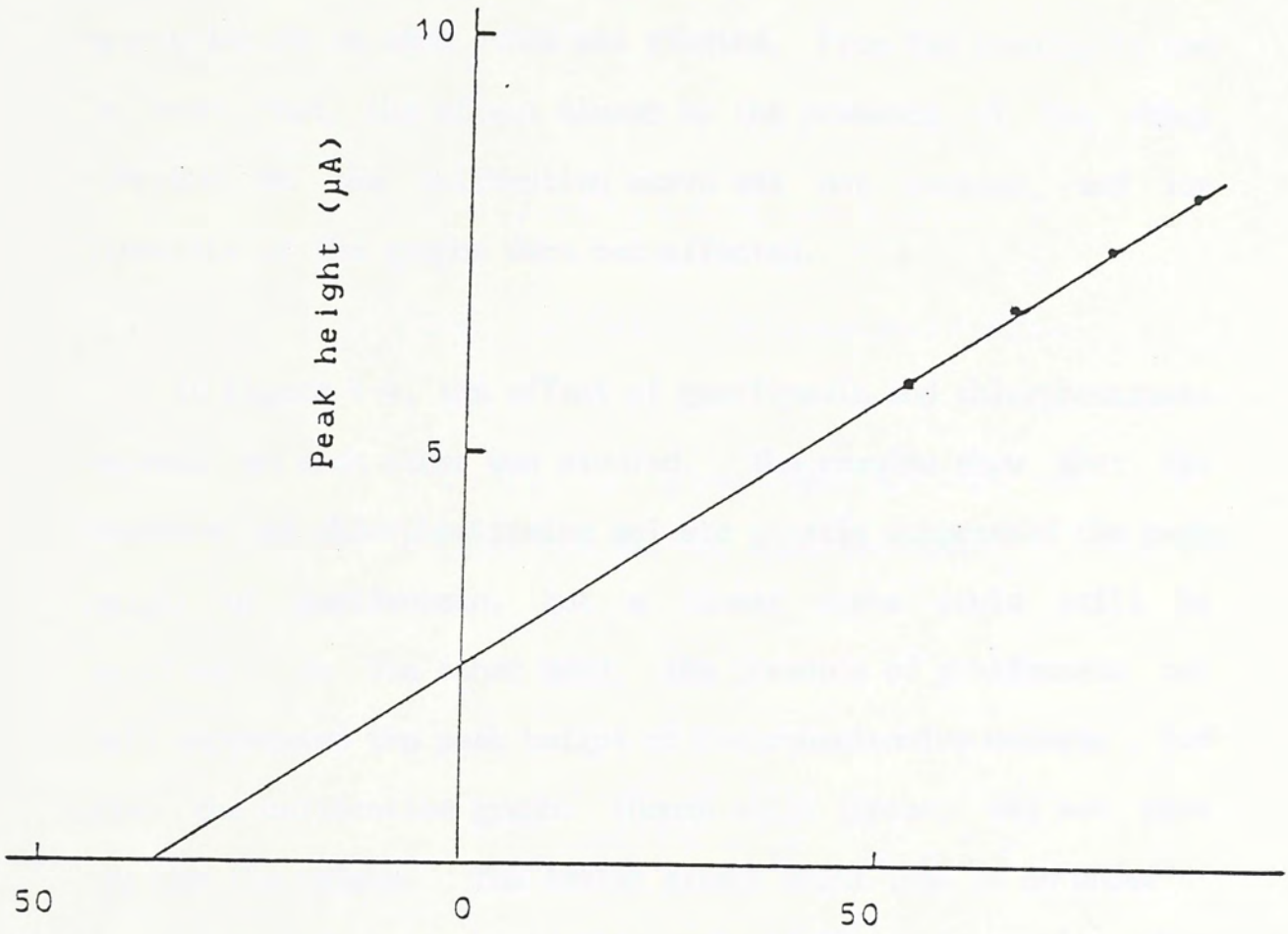


Figure V-2 : Standard addition curve in determining chlorpheniramine maleate in the presence of a synthetic mixture of excipients

ingredients on each other would be assessed and the results are summarised in the Figures V-3 & V-4.

In Figure V-3, the effect of guaifenesin and phenylephrine hydrochloride on each other was studied. From the result, it can be seen that the effect caused by the presence of the other compound on the calibration curve was not serious, and the linearity of the graphs were not affected.

In Figure V-4, the effect of guaifenesin and chlorpheniramie maleate on each other was studied. The results show that the presence of chlorpheniramine maleate greatly suppressed the peak height of guaifenesin, but a linear curve could still be obtained. On the other hand, the presence of guaifenesin not only suppressed the peak height of chlorpheniramine maleate , but also the calibration graph, though still linear, did not pass through the origin. The latter effect might lead to an under - estimation of chlorpheniramine maleate in real samples when applying the standard - addition calibration.

The peak potentials of phenylephrine hydrochloride and chlorpheniramine maleate are + 0.560 V and + 0.675 V versus S C E, respectively. The two peaks are so close that overlap of these peaks occur very often.

It was found in this study that the presence of 10.2 ppm chlorpheniramine maleate would cause a 4.9 % lowering of peak height of 9.2 ppm phenylephrine hydrochloride. On the other

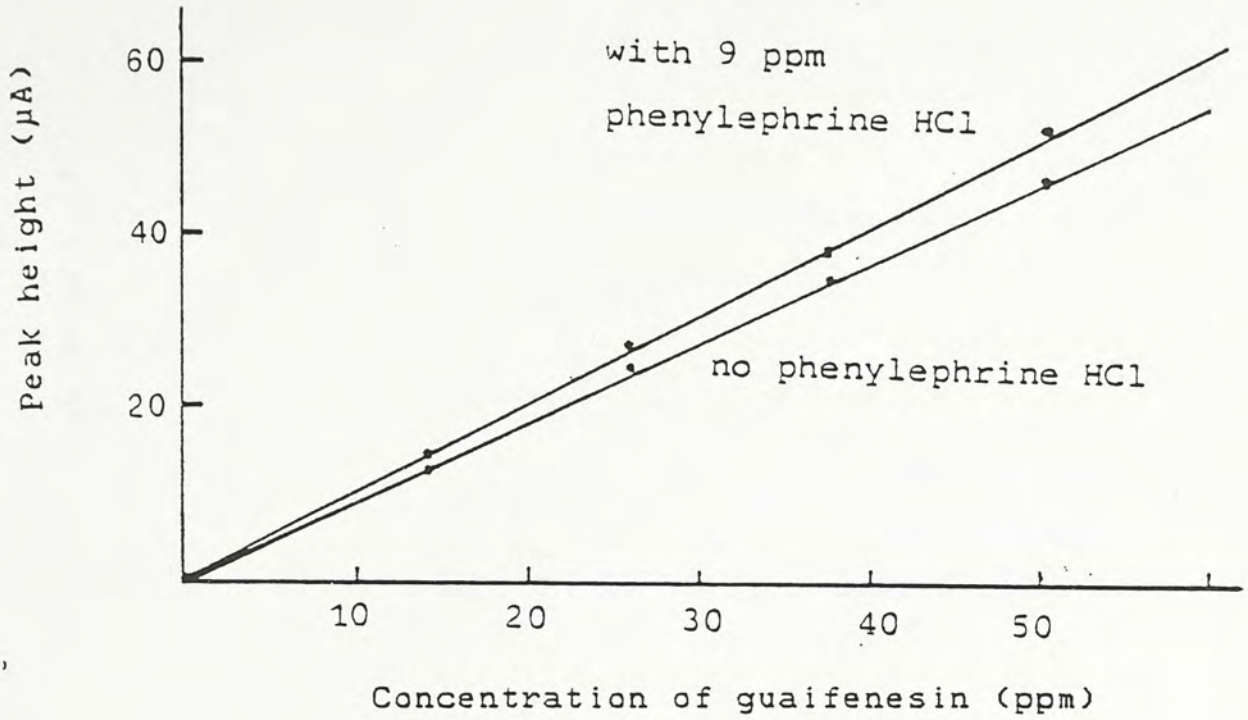


Figure V-3 (a) : Effect of phenylephrine hydrochloride on the peak height of guaifenesin

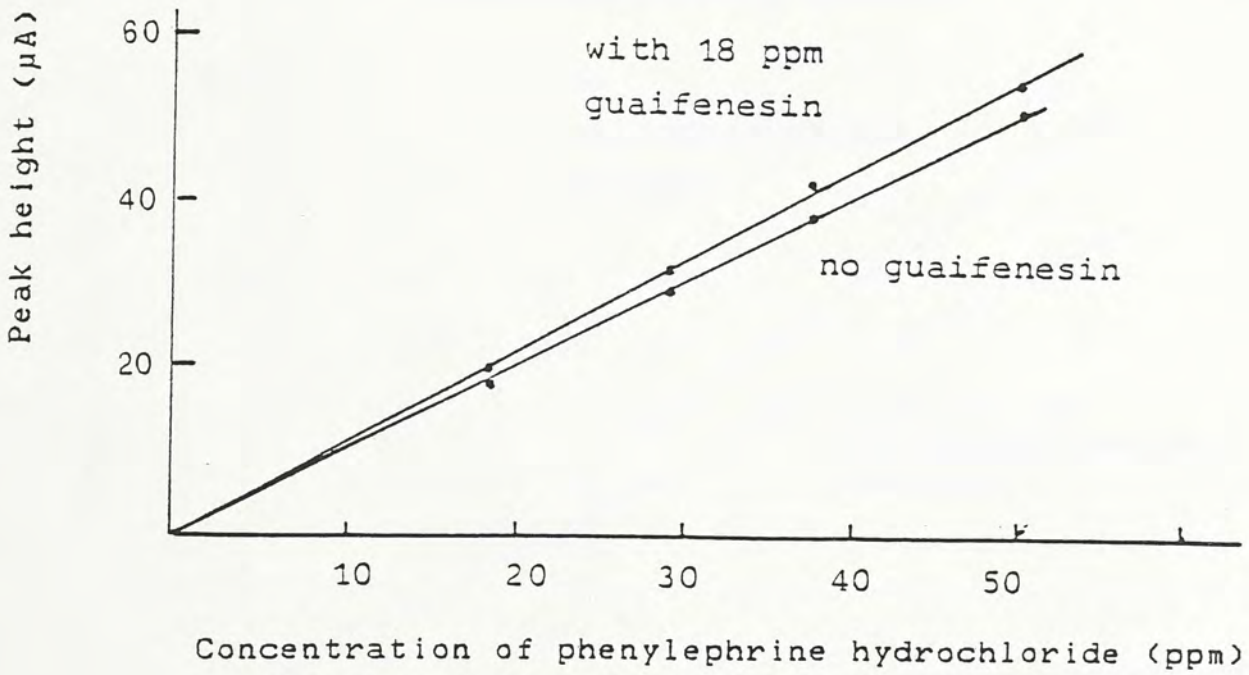


Figure V-3 (b) : Effect of guaifenesin on the peak height of phenylephrine hydrochloride

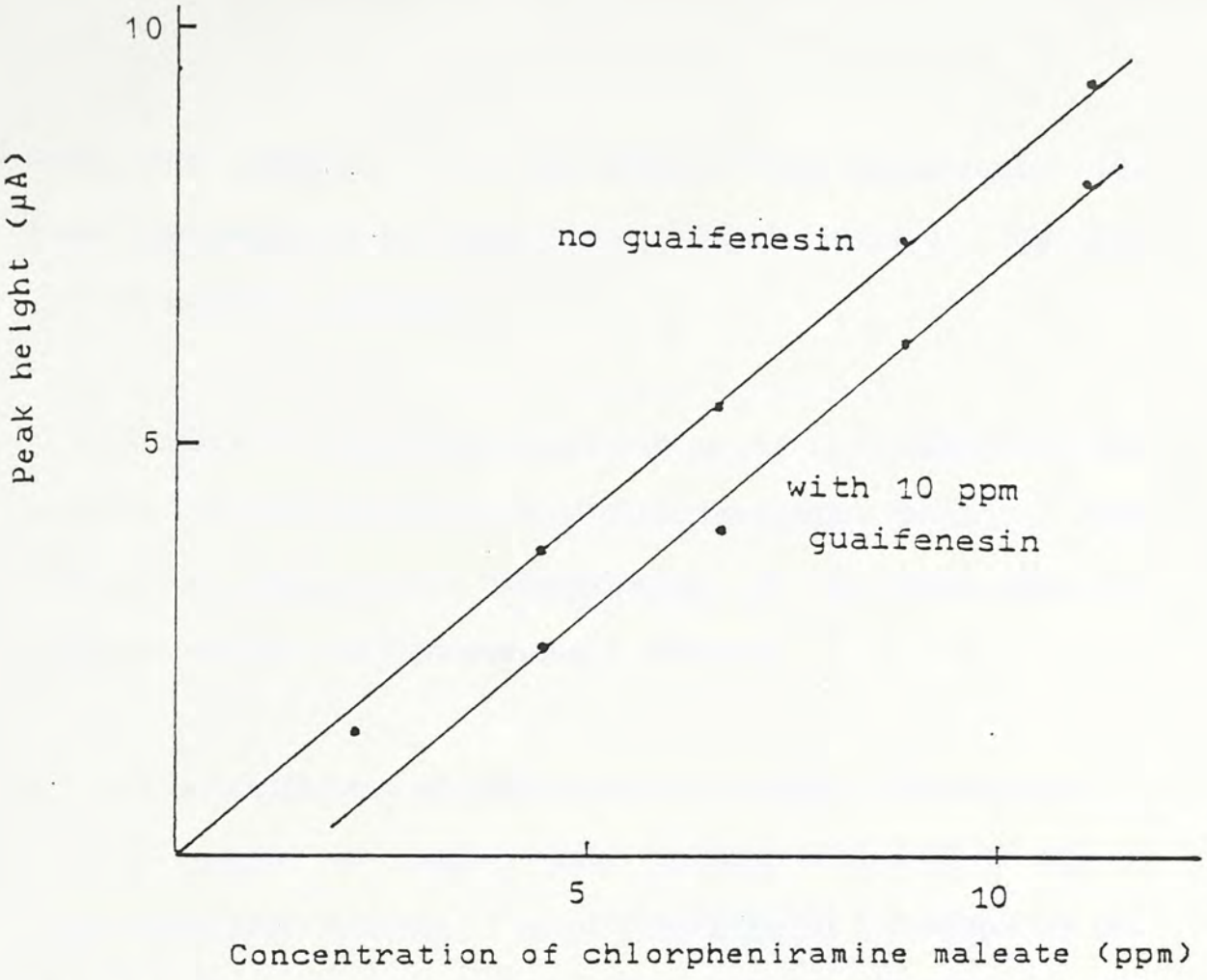


Figure V-4 (a) : Effect of guaifenesin on the peak height of chlorpheniramine maleate

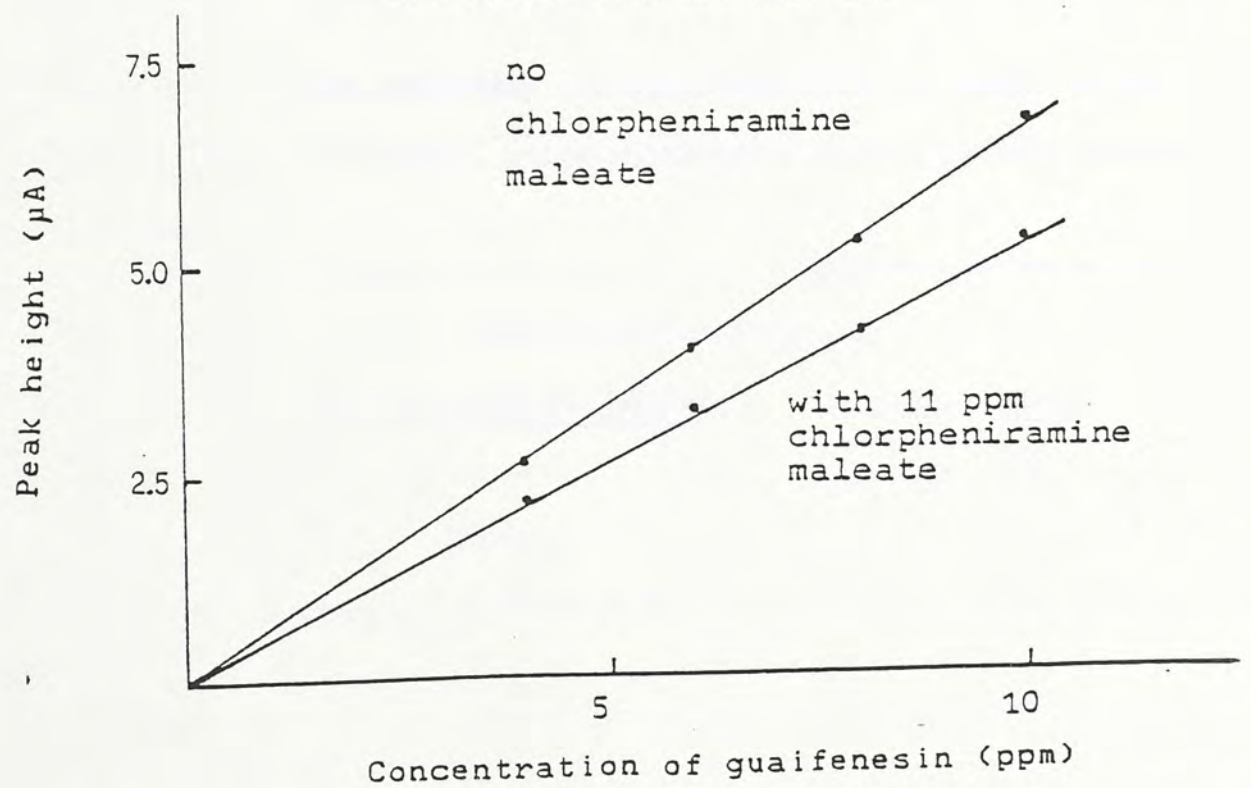


Figure V-4 (b) : Effect of chlorpheniramine maleate on the peak height of guaifenesin

hand, the presence of 0.9 ppm phenylephrine hydrochloride was found to cause a 4.5 % lowering of the peak height of 32.6 ppm chlorpheniramine maleate.

Because of the mutual interference, it is expected that the accuracy of the determination of chlorpheniramine maleate in the presence of phenylephrine hydrochloride, or vice versa using the proposed method would be seriously affected.

(3) The Determination of Guaifenesin in a Cough - Cold mixture

A sample of cough - cold mixture contained 1 mg of chlorpheniramine maleate, 5 mg of phenylephrine hydrochloride and 25 mg of guaifenesin in 5 ml of solution. Results of the determination of guaifenesin in this are shown in Table V-4.

Table V-4 : Determination of guaifenesin in a cough - cold mixture by differential pulse voltammetry with a glassy carbon electrode

	Guaifenesin content (mg)	
	By voltammetric method *	label
Trial 1	23.4	25
	21.0	
average :	22.2 (7.6 %)	
Trial 2	24.0	25
	21.3	
average :	22.7 (8.4 %)	

* relavtive standard deviation quoted in parentheses

The results show that the amount of guaifenesin found by the proposed voltammetric method differed from the label value by about 10 %. Thus, it may concluded that the proposed voltammetric method in cough - cold mixtures cannot be applied for the simultaneous determination of active ingredients. It is also not very satisfactory to determine one ingredient in the presence of other active components in such mixtures.

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Appendix : THE SIMULTANEOUS DETERMINATION OF FIVE COMMON ACTIVE COMPONENTS IN COUGH MIXTURES BY THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The simultaneous determination of the active components in a cough mixture has often been done by chromatographic method. Simultaneous gas liquid chromatography was successful in assay for amine (1) (2). However, the gas liquid chromatographic methods were generally less efficient and it may require derivatization of some or all amines.

The application of high - performance liquid chromatographic (HPLC) procedure to various different kinds of combinations of components had been reported (3) (4). The reverse - phase, ion - paired H P L C was often used and some attention had been given to the effect of ion - pairing agents (5) (6).

In the following study, the analysis of phenyephrine hydrochloride, codeine phosphate, ephedrine hydrochloride, chlorpheniramine maleate and phenylpropanolamine hydrochloride are performed utilizing an isocratic reverse - phase, ion - paired high - performance liquid chromatographic system.

MATERIAL

All active ingredients were of B P quality and were used without further purification.

MOBILE PHASE

The content of the mobile phase is illustrated as followed :

Methanol	680 ml
Water	290 ml
Phosphoric acid (85 %)	1 ml
Sodium dioctyl sulfosuccinate	5.8 g
Tetrahydrofuran	40 ml
pH (adjusted by ammonium hydroxide)	4.6

INSTRUMENTATION

The chromatogram conditions are listed in the following table.

(a) Chromatograph *

Column	C 18 (7 um particles)
Flow rate	1.3 ml/min
Pressure	3500 psi
Temperature	ambient

(b) Detector **

Wavelength	254 nm
Slit width	2 nm
Aufs	0.05
Chart speed	15 mm/min

* Perkin Elmer Series 2 Liquid Chromatograph

** Hitachi 220 Spectrophotometer

INTERNAL STANDARD SOLUTION

Metoclopramide hydrochloride was dissolved in the mobile phase to make up solution of 0.0372 mg/ml

PROCEDURE

About 35 ul of sample or standard solution was chromatographed. The concentration of the sample was determined from the calibration of the peak height ratio relative to the internal standard peak.

RESULTS AND DISCUSSION

In choosing the composition of the mobile phase, methanol and water are the main components and a study of the effect of methanol on the retention time has been studied.

	Retention time (min)		
Methanol content :	67 %	76 %	78 %
Codeine phosphate	5.2	3.9	3.4
Ephedrine HCl	6.7	4.7	4.5
Chlorpheniramine maleate	16.5	9.0	6.6

The increasing of the methanol content would decrease the retention time of the ingredients, as shown by that of chlorpheniramine maleate. This shorter retention time would decrease the chromatographic run time. However, the resolution between ephedrine hydrochloride and codeine phosphate was decreased and overlapping was observed. Therefore, a mobile

phase containing 67 % methanol and 4 % tetrahydrofuran (which reduces tailing of analytical peaks) was chosen.

In the experiments, sodium dioctyl sulfosuccinate was used as the pairing - ion. There was a detailed study of the effect of the carbon chain length on pairing ion (4), and it was found that the dioctyl sulfosuccinate ion was the most suitable after considering the solubility, availability and cost. Chromatograms of our study also showed that this pairing ion possessing the ability to produce adequate retention of the ingredients.

In the mobile phase, the phosphate buffer was used and the required pH value was adjusted by adding ammonium hydroxide.

The pH effect on the retention time is illustrated in the following table.

	pH 4.6	pH 3.8
Codeine phosphate	5.3	7.1
Ephedrine hydrochloride	7.0	9.3
Chlorpheniramine maleate	19.0	27.0

The retention time of chlorpheniramine maleate was rather long at pH 3.8, the reason would be that the two nitrogen atoms were protonated and the retention time was thus long. However, at pH 4.6, only the tertiary amine group was protonated so the retention time was shorter. Meanwhile, a pH of 4.6 could provide a sufficient resolution between codeine phosphate and ephedrine hydrochloride.

On the other hand, the flow rate of the mobile phase would also affect the retention of the ingredients.

Flow rate :	1.3 ml/min	1.5 ml/min
Codeine phosphate	5.3	5.2
Ephedrine hydrochloride	7.0	6.7

By increasing the flow rate, the retention time of the ingredient would be shortened, but the back pressure would increase. The back pressure of about 3800 psi results with the flow rate at 1.5 ml/min, and may cause damage to the pump. The slower flow rate was chosen throughout the experiment.

During the quantitative determination of samples, the concentrations of the ingredients were calculated from the peak height relative to that of the internal standard peak. Therefore, a suitable internal standard was screened. A number of related compounds has been tried and their retention times are shown in the following table.

Table : The retention times of some organic compounds

Compound	Retention time (min)
Guaifenesin	2.5
Caffeine	2.6
Procaine hydrochloride	4.7
Pseudoephedrine hydrochloride	6.5
Metoclopramide hydrochloride	8.0
Pheniramine maleate	1.8 & 9.6
Scopolamine - N - butylbromide	9.9
Doxylamine succinate	11.3
Benzphetamine hydrochloride	11.7
Diphenyhydramine hydrochloride	16.5
Dextromethorphan hydrobromide	17.1
Bromhexin hydrochloride	30.0

After considering with the retention time, and the peak shape of the above compounds, metoclopramide hydrochloride was chosen as the internal standard.

The five ingredients of interest can be divided into two groups according to their relative retention time to that of metoclopramide :

Mixture I	Retention time (min)	Retention time ratio
Phenylephrine hydrochloride	4.7	0.57
Phenylpropanolamine hydrochloride	7.0	0.84
Chlorpheniramine maleate	18.0	2.16
Metoclopramine hydrochloride	8.3	1.00

Mixture II		
Codeine phosphate	5.3	0.64
Ephedrine hydrochloride	7.0	0.84
Chlorpheniramine maleate	19.0	2.30
Metoclopramine hydrochloride	8.3	1.00

It is noted that guaifenesin was not included in the study, because the peak of guaifenesin is always masked by those of common excipients.

The calibration of each ingredient is plotted with the peak height ratio versus the concentration using 0.0372 mg/ml metoclopramide hydrochloride as the internal standard. The calibration curve of components in mixture I are shown in Figure A-1 to A-3.

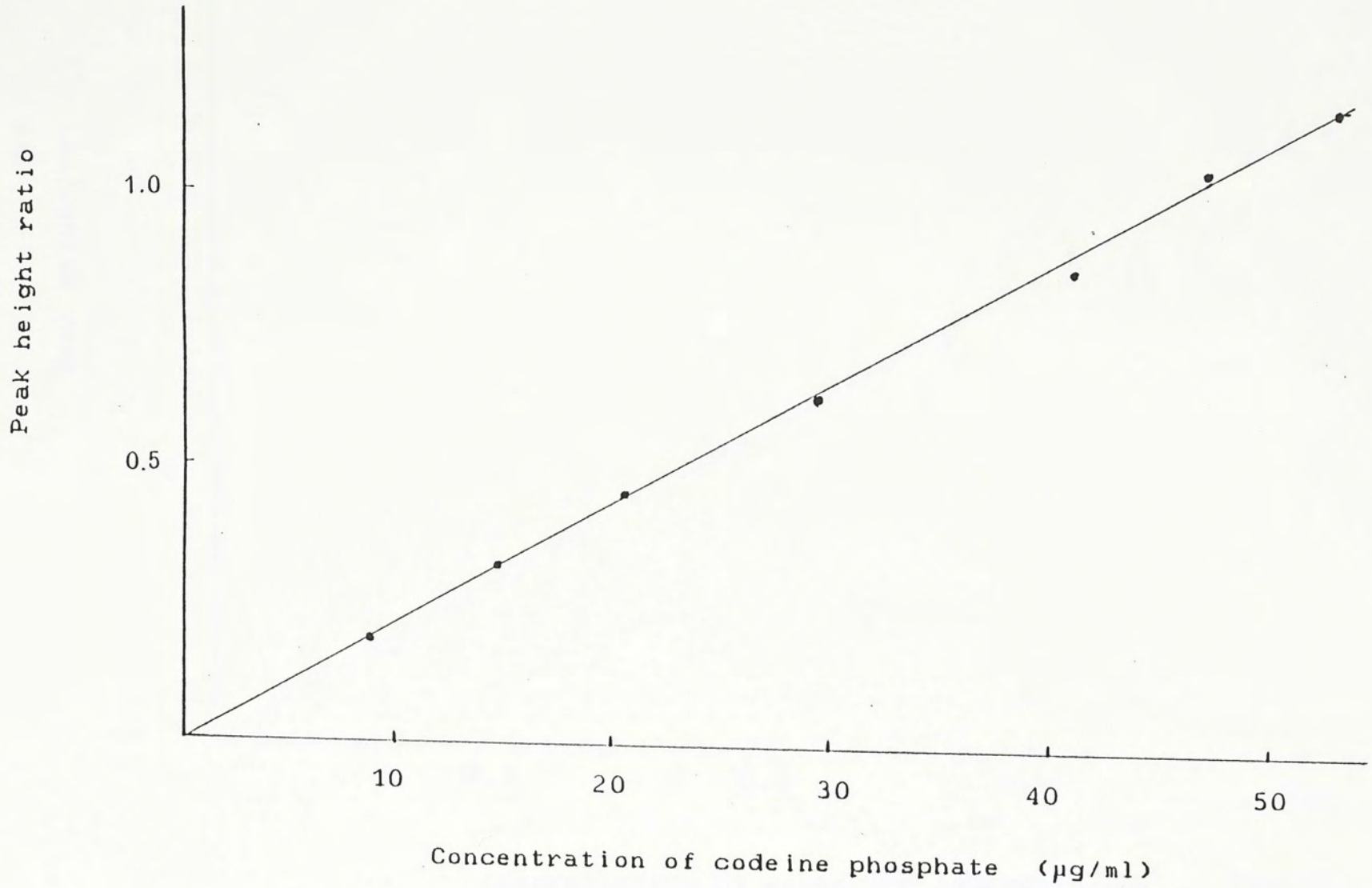

Figure A-1 : Calibration curve of codeine phosphate

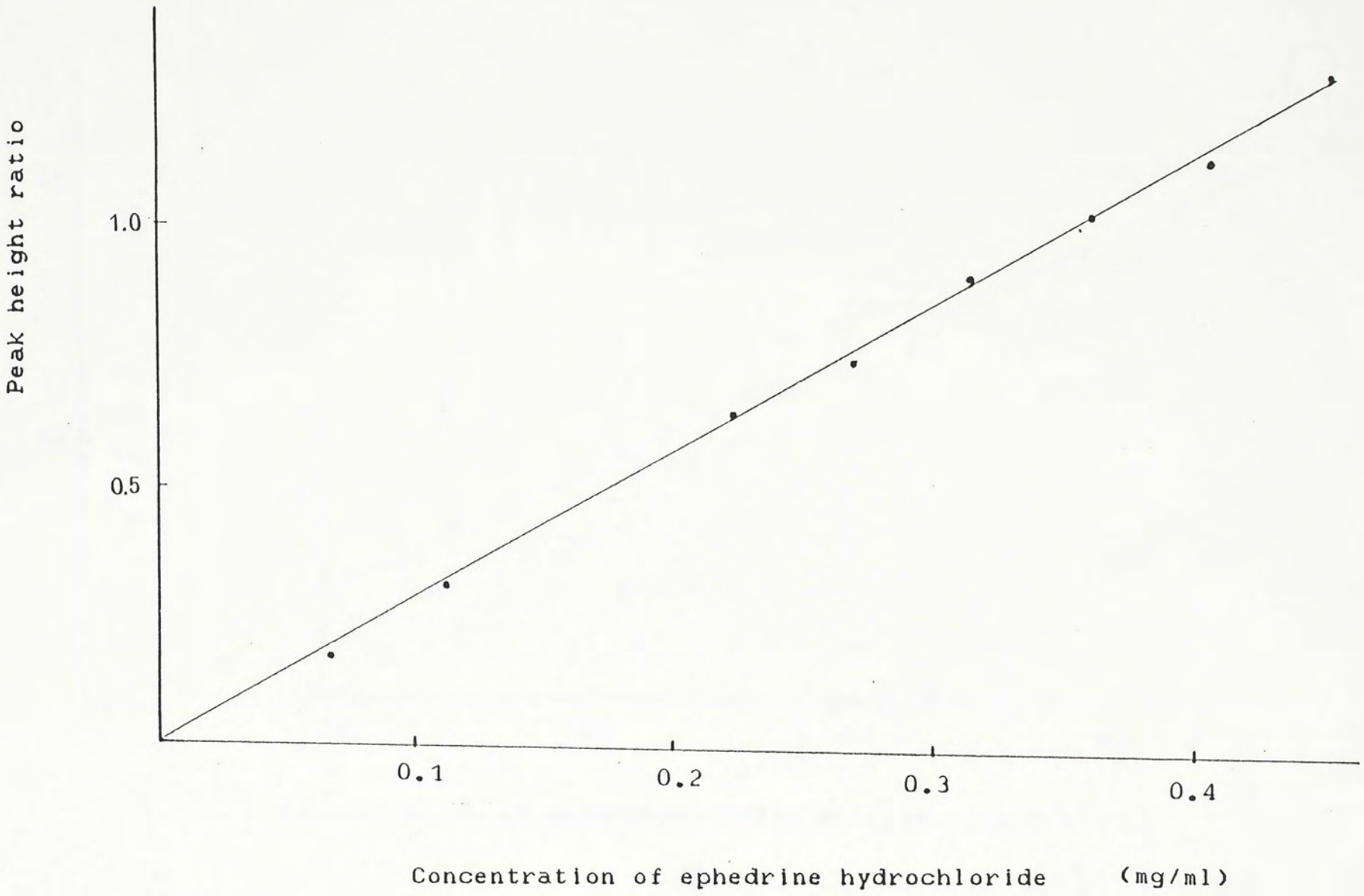

Figure A-2 : Calibration curve of ephedrine hydrochloride

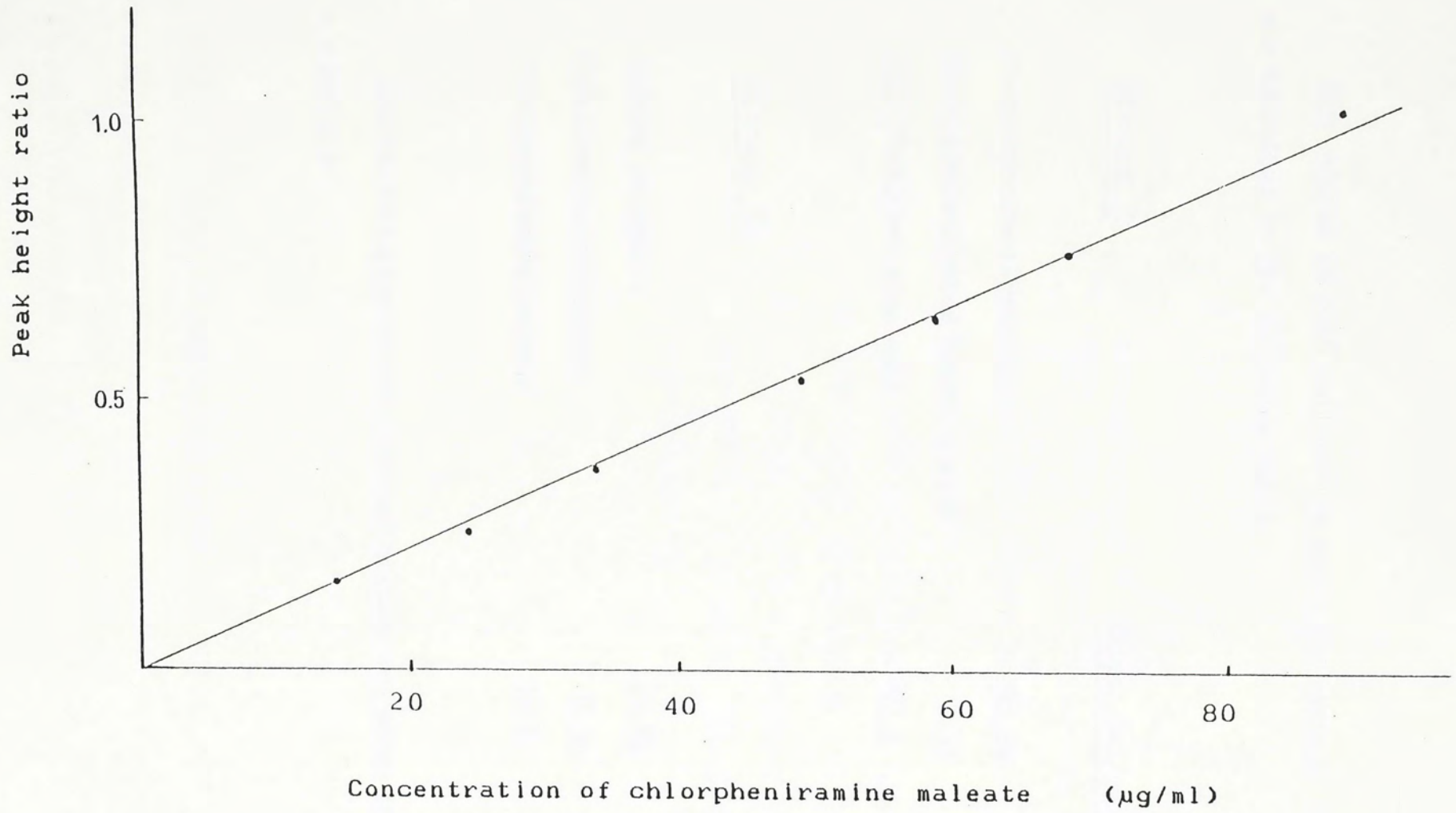

Figure A-3 : Calibration curve of chlorpheniramine maleate

The slopes of each calibration curve for various compounds are tabulated in the following table.

Mixture I	slope (ml/μg)
Phenylephrine hydrochloride	92.59
Phenylpropanolamine hydrochloride	0.33
Chlorpheniramine maleate	92.3

Mixture II	
Codeine phosphate	45.71
Ephedrine hydrochloride	0.36
Chlorpheniramine maleate	93.6

The chromatogram of the standard mixture are shown in Figure A-4 and A-6.

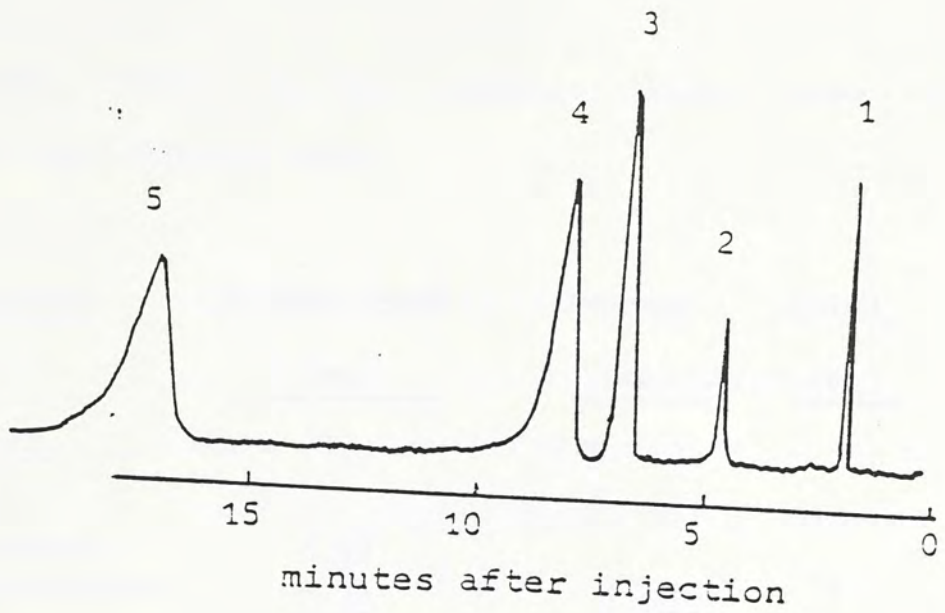

Figure A-4 : Chromatogram demonstrating the retention behaviour of ingredients in mixture I : maleic acid (1); phenylephrine (2); phenylpropanolamine (3); metoclopramide , internal standard (4); and chlorpheniramine (5)

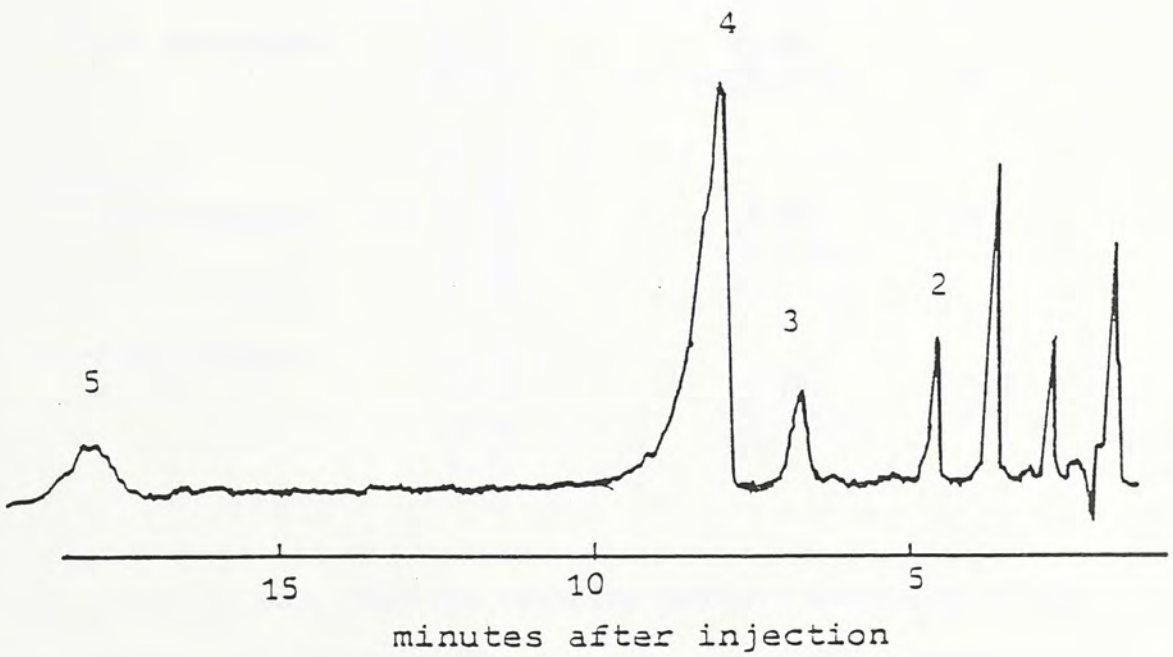

Figure A-5 : Chromatogram of commercial mixture I

The assay results for two commercial dosage forms are presented in the following table.

Ingredients	Weight found (mg)	Average (mg)	Label (mg)
Sample I			
Phenylephrine hydrochloride	4.89 4.91 4.92	4.91 (0.3 %)	5
Phenylpropanolamine hydrochloride	4.64 4.75 4.79	4.73 (1.6 %)	5
Chlorpheniramine maleate	3.63 3.51 3.67	3.60 (2.3 %)	4
Sample II			
Codeine phosphate	11.69 12.03	11.86 (2.0 %)	12
Ephedrine hydrochloride	5.85 5.77 5.89 5.77	5.82 (1.0 %)	6
Chlorpheniramine maleate	1.13 1.13 1.16 1.21	1.18 (3.3 %)	1.2

The results show that the relative standard deviation of the weight found for each ingredient are less than 2 % except for chlorpheniramine maleate. In fact, the deviation of the chlorpheniramine maleate is relatively large because of the long

retention time of the peak so that the shape of the peak is board. Thus, the peak height could not be estimated accurately.

The correponding chromatograms of the sample are shown in Figure A-5 and A-7. It can be seen from the chromatograms that none of the excipients in the formulation interferes with the analytical peaks of active ingredients or the internal standard.

CONCLUSION

The reverse-phase, ion-pair high-performance liquid chromatographic method has been developed for the simultaneous determination of active ingredients in cough-cold mixtures. Excipients produced no interference to the estimation of any of the compounds; so pre-treatment of the sample was unnecessary. Moreover, the sample preparation only requires the dilution with the mobile phase. The method is found to be suitable for the simultaneous determination of some active ingredients in cough mixtures commonly available.

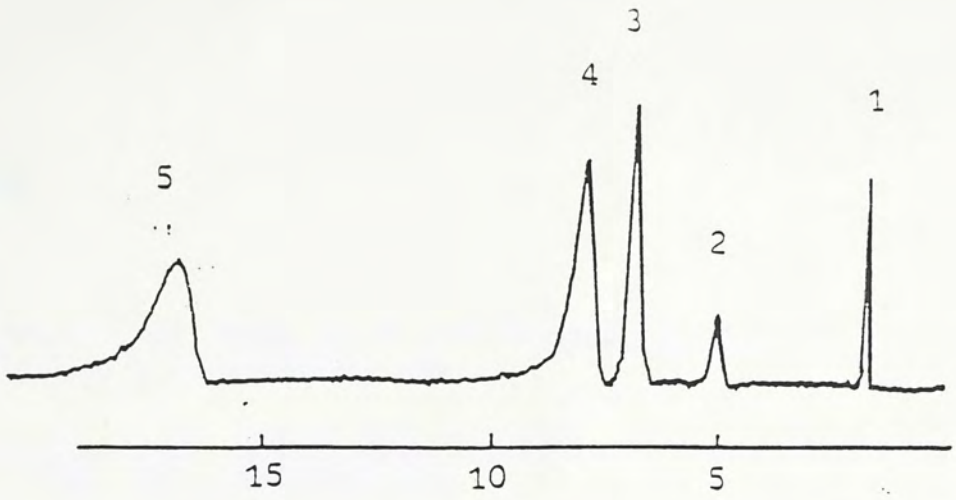

Figure A-6 : Chromatogram demonstrating the retention behaviour of ingredients in mixture II : maleic acid (1); codeine (2); ephedrine (3); metoclopramide, internal standard (4); and chlorpheniramine (5)

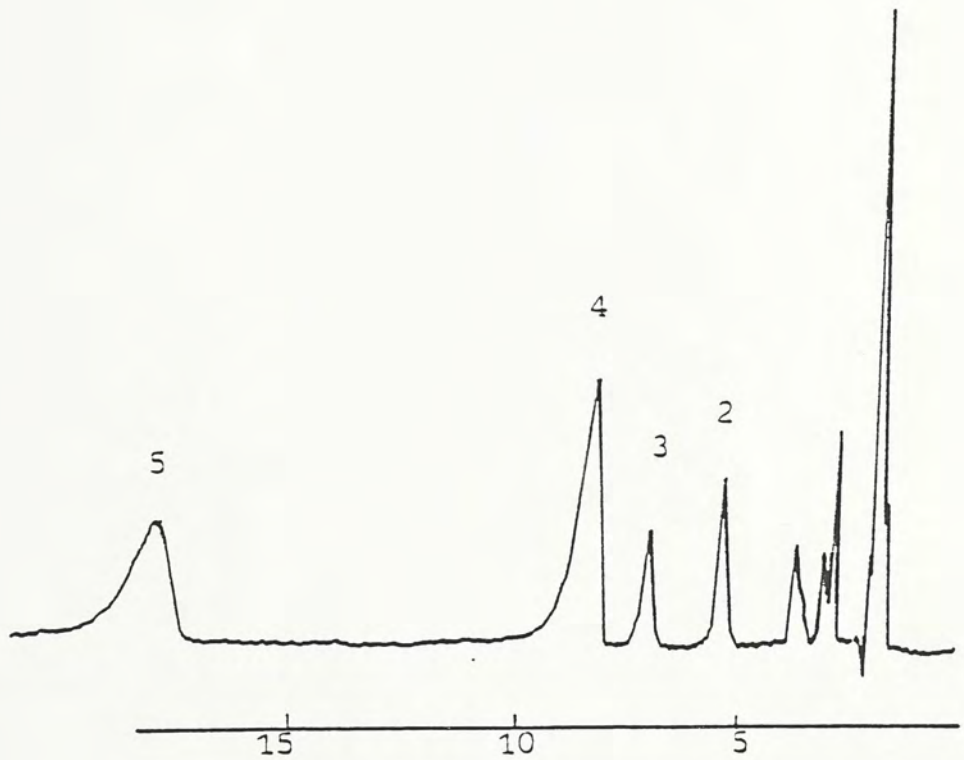

Figure A-7 : Chromatogram of commercial mixture II

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